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ON PAIRS OF SPECIES

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(WITH TWELVE FIGURES)

Our growing knowledge of the definiteness of variation makes it desirable that botanists should begin to apply this knowledge to a more detailed study of the relationships of particular species. With this end in view I have endeavored to begin such a study by the examination of the various relationships between pairs of species in the same genus. Every botanist knows numbers of such cases, and it occurred to me that it would be worth while to analyze several such pairs as regards their differential characters, habitats, and distribution, to discover whether any light can be thrown in this way on the probable origin of the species in question. How have these differences arisen in the light of our present views of variation?

Again, various rules of distribution have been proposed, such as JORDAN'S law that related species occupy adjacent areas. It is not the purpose of this paper to discuss questions of distribution at any length, but it will be seen incidentally that a species and its next of kin may occupy (1) the same locations, (2) adjacent areas, or (3) widely separated regions. They may overlap, or the distribution may be practically coterminous. From such facts as these it might appear that JORDAN'S law as applied to plants is more honored in the breach than in the observance; but when applied to the variations or tendencies to variation within the taxonomic species, every botanist knows how usually it holds as modified by

topographical conditions. In passing from east to west or from north to south of the continent, the succession of species often seems to form a graded series, though more or less disturbed and modified by the incidence of mountain ranges, plains, etc., a fact which is relied upon in many ways by systematic botanists in their pursuits.

This preliminary study of the relationships and distribution of pairs of species is perhaps of more value for its suggestiveness than for any direct contribution of facts; at least it is to be hoped that such is the case, for the species I have chosen are all familiar forms in the North American flora. Nevertheless, from the material I have examined in this study it has been necessary to describe several new and hitherto unrecognized varieties, and certain others will be described in another connection.

Notwithstanding the great amount of speculation, and more recently of experimental work, on the factors of evolution, scarcely any attempt has been made hitherto to show how one living wild species has been derived from another particular species, or from the common ancestor of both. No doubt systematists frequently have such questions in mind when delimiting species, but, if the methods of experimental evolution are sound, they should enable us by now to begin the application of the ideas so gained to the solution of simple examples taken from wild nature. Even though the historical relationships of many species must remain obscure, yet there exist cases in which the course of events is simple and to some extent within our present powers of analysis.

The pairs I have chosen have been taken at random. In a subsequent study I may make a more methodical selection. In some instances of species pairs the genus is bitypic; in others the two species may stand apart from the others in the genus, either in their structure or their distribution. Some of the cases of real pairs, however, are not obviously pairs at all, and are only found to be such by a study of their internal structure. On the other hand, some species which form an apparent pair in a given region are not very closely related to each other, and have only become paired through the vicissitudes of altering distributions. Such instances show that the mere taxonomic comparison of species, un-

less supplemented by histological or experimental investigations, may lead to quite erroneous ideas concerning the relationships of the species within a genus. There needs to be developed a taxonomy based upon the anatomical and cytological structure of plants, as well as upon the traditional comparison of their external morphology. This need has often been emphasized, and it appears now to be time to begin to put the principle into practice. If the present paper leads to the closer scrutiny of known species from this aspect its purpose will have been accomplished.

An instructive example of the value of cytology in determining relationships has recently been furnished by *Spiranthes* (*Gyrostachys*) *cernua* (L.) Richard. Reasoning by analogy from the case of *Oenothera gigas*, Miss PACE[1] found that it is a true cell giant, having twice as many chromosomes as *S. gracilis* (Bigel.) Beck, and a corresponding increase in cell size and stature. The two species are shown in fig. 1. *S. cernua* is conspicuously larger in all its parts, having larger flowers, stouter stems, and longer, though usually narrower, leaves.[1a] There is variation particularly in the length of the spike and the width and shape of the basal leaves.

ANDREWS[2] observed three distinguishable forms of *S. cernua* in a meadow at Williamstown, Massachusetts. The type is pure white and fragrant. A variety which was the common form in this meadow differed in having cream colored or yellow flowers which were not fragrant, a shorter, broader, and more rounded or 2-lobed lip, and leaves also distinct in shape and structure. A second variety, found in one spot some distance away, was white flowered, but otherwise agreed with the yellow variety. Similar forms are recorded from Manchester, New Hampshire, and from Mount Desert Island, Maine. A var. *ochroleuca* (Rydb.) Ames has also been described, having greenish, cream colored or white flowers, longer floral bracts, growing in dry ground, and blooming somewhat later. The tetraploid *S. cernua* would thus appear

[1] In *S. gracilis* $2x = 30$, while in *S. cernua* $2x = 60$. PACE, LULA, Two species of *Gyrostachys*. Baylor Univ. Bull. 17. no. 1. pp. 16. *figs.* 50. 1914.

[1a] Figures of these species are also found in TORREY, Fl. N.Y. **2**: 282. *pl.* 129. 1843.

[2] ANDREWS, LEROY, On some variations of *Spiranthes cernua*. Rhodora **1**: 110–111. 1899.

to be more variable than other species, such as *S. gracilis* and *S. praecox*. Indeed, it is stated in GRAY's *Manual* to be "very variable in size and foliage." This is interesting because of the fact that *Oenothera gigas* is also more variable, particularly in foliage, than the other, non-tetraploid, mutants. The increased variation, which is of a remarkable kind in *O. gigas*, is probably concerned with new distributions of the quadruple chromosome series in meiosis.

FIG. 1.—*Spiranthes gracilis* (three plants on the left) and *S. cernua* (plant on the right).

Another interesting feature of *S. cernua*, which apparently has not been reported in the other species of *Spiranthes*, is the prevalence of apogamy and polyembryony. LEAVITT[3] found that an abundance of seed is set when fertilization is excluded, and that 1–5 or 6 adventive embryos occur. They vary greatly in shape, from

[3] LEAVITT, R. G., Polyembryony in *Spiranthes cernua*. Rhodora **2**:227–228. 1900.

———, Notes on the embryology of some New England orchids. Rhodora **3**:61–63, 202–205. *pl. 33*. 1901.

spherical to elongate, irregular, or lobed. In a few plants of this species the embryo sac develops normally, followed by fertilization and the production of embryos, but the embryos always possess an apical protuberance which is lacking in the polyembryonic embryos of apogamous plants. The exact manner of origin of the apogamous embryos was not determined, but it appears that individual plants produce embryos which are either all apogamous or all resulting from fertilization. This matter is worthy of further study. No other orchid is known to exhibit this type of polyembryony, although twin embryos occur in many species; but the latter are believed to result from a doubling of the embryo sac followed by fertilization by two pollen tubes. These facts are of interest because it is known that tetraploid species are frequently apogamous.[4]

It has also been observed[5] that a peculiar form of vegetative multiplication takes place in *S. cernua*, in which young plants are produced from the root tips, but in this case a similar development was reported by STRASBURGER in *Neottia* sp.

Another interesting point is the manner in which light is thrown on relationships by cytological study. The mutation theory is destined in this way to modify many current taxonomic conceptions of relationship. *Spiranthes cernua* in the manuals is separated from *S. gracilis* by several other species, yet it must have been derived at some time from this or possibly from one of the other diploid species. It is even possible, as Miss PACE suggests, that *S. cernua* may still be arising by sporadic mutations from *S. gracilis*. Both species have much the same range, from Nova Scotia to Manitoba, Florida, and Texas. The borders of distribution of this pair of species are nearly, if not quite, coterminous.

CLINTONIA BOREALIS (AIT.) RAF. AND C. UMBELLULATA (MICHX.) TORR.

As regards distribution, *Clintonia borealis* is more northerly and much more widely distributed than *C. umbellulata*. It occurs

[4] See GATES, R. RUGGLES, The mutation factor in evolution. London: MacMillan and Co. 1915 (pp. 197 ff.).

[5] HALL, J. G., Vegetative reproduction in *Spiranthes cernua*. Rhodora 7:40–50. *fig. 1.* 1905.

from Newfoundland to Minnesota, and south in the mountains to North Carolina. *C. umbellulata*, on the other hand, is confined to the region from New York to Georgia and Tennessee. A comparison of the species shows the following differences:

C. borealis[6] (fig. 2)	*C. umbellulata* (fig. 3)
Scape 1.5–4 dm. high	Scape 2–4.5 dm. high
Umbel 3–6-flowered, pedicels stouter	Umbel several to many-flowered, pedicels shorter, pubescent
Flowers greenish-yellow, 8–10″ long	Flowers white, odorous, often purple dotted, 4–5″ long
Ovules numerous, in 2 rows in each cavity	Ovules 2 in each cavity
Berry oval, several-seeded	Berry globose, few-seeded

There is apparently no constant difference in the foliage or pubescence, though *C. umbellulata* frequently has larger leaves and is usually more pubescent. *C. umbellulata* has, on the average, a somewhat taller scape; its flowers are more numerous but only half as large as in *C. borealis;* the pedicels are also shorter, more slender, and more pubescent, and the ovules and seeds fewer. In both species the leaves have ciliate margins. In *C. borealis* the scape is nearly glabrous, the pedicels more pubescent; in *C. umbellulata* the scape is pubescent and the pedicels densely so. Summing up the differences, we find them chiefly quantitative, and yet the species do not overlap and there is never any difficulty in distinguishing them, unless it be in the region where they both occur. Here it is possible that there may be intercrossing, giving rise to intermediate forms; but it seems clear, as BATESON[7] has pointed out in similar cases, that such intermediates are, at least in many instances, secondary and not primary in origin. They appear where the two species come in contact, and result from crossing rather than from original variations.

Although *C. umbellulata* averages larger in size, its flowers are conspicuously smaller, and white in color. It is evident that all

[6] In drawing up the contrasting characters I have frequently consulted GRAY's *Manual* and BRITTON and BROWN's *Flora*.

[7] BATESON, WM., Problems of genetics. p. 158 and elsewhere. Yale University Press. 1913.

of these changes could not have arisen through a single mutation, so it becomes necessary to postulate a common ancestor for the two species. Such an ancestor we may suppose threw off a series of

FIG. 2.—*Clintonia borealis* (Ait.) Raf.

mutations which again continued to mutate in new directions, as we know to happen in other forms from genetic experiments. The surviving forms which we now know as C. *borealis* and *C. umbellulata* might easily represent a differential of not more than three or

FIG. 3.—*Clintonia umbellulata* (Michx.) Torr.

four mutations. The various other mutants and combinations we may suppose to have been eliminated by selection or by their own instability. The fact that *C. umbellulata* is odorous, while *C. borealis* has no marked odor, is by no means unique. Similar cases occur in various other genera, including *Oenothera* and the variation in *Spiranthes cernua* recently mentioned. They find their parallel and no doubt their basis in organic chemistry, where a change of an atom or even a rearrangement of the atoms in a molecule produces an odorous compound from an odorless one.

Streptopus amplexifolius (L.) DC. and S. roseus Michx.

In comparing these well known species we find a more marked series of differences. As regards distribution, *S. amplexifolius* is boreal and circumpolar, occurring in Europe and Northern Asia, Greenland, Newfoundland, Labrador to Alaska, and south to North Carolina and California. *S. roseus* is not found in Europe or Asia, but occurs from Newfoundland and Labrador to Alaska, and southward to North Carolina and Oregon.

Fernald[8] has made a careful study of the differences between the two species, which may be briefly set forth as follows:

S. amplexifolius (fig. 4)	*S. roseus* (fig. 5)
Stem whitish and glabrous above	Stem greenish and usually ciliate-hispid above
Leaves strongly glaucous, amplexicaul, glabrous	Leaves green, scarcely amplexicaul, conspicuously ciliate
Perianth segments spreading widely and quickly recurved	Perianth segments slightly divergent, only the tips becoming recurved
Anthers lance-subulate, entire, many times longer than the filaments	Anthers narrow-ovate, bifid, about the length of the filaments
Stigma subentire or merely shallow-lobed	Stigma deeply 3-cleft

These two common and widespread species thus exhibit a number of conspicuous unit differences, which are unlike the differences in the pair of species of *Clintonia* previously examined. The

[8] Fernald, M. L., The genus *Streptopus* in eastern America. Rhodora **8**:69–71. 1906.

FIG. 4.—*Streptopus amplexifolius* (L.) DC.

FIG. 5.—*Streptopus roseus* Michx.

most conspicuous of these differences would appear to be probably quite independent of each other, and we cannot imagine them all having originated at one stroke. Thus, the leaves of one are (1) glaucous and amplexicaul, of the other beautifully ciliate; (2) the perianth segments of one are widely spreading and recurved, of the other campanulate; (3) the anthers of the one are entire on long filaments, of the other forked and on short filaments; (4) the stigma of the one is nearly entire, of the other 3-cleft. These four main differences are probably not correlated with each other, and may have originated through several independent changes in the common ancestor. This hypothetical ancestor we may suppose threw off a series of new forms differing from each other in various unit characters, just as mutations are known to occur in *Oenothera*, *Drosophila*, and other genera today. The forms exhibiting these unit differences intercrossed, and, certain of the resulting combinations proving more stable than others, two of the more extreme combinations finally survived, while the others gradually disappeared. This is of course only one of the possible hypotheses to account for the occurrence of two such species.

A hybrid between these two species has been described by FERNALD[9] from the Gaspé Peninsula under the name of *S. oreopolus*. This form has leaves less ciliate than in *S. roseus*, and flowers like those of *S. amplexifolius* but deep claret-purple in color. There is thus some evidence that the various character differences do behave independently of each other, and it is also a significant fact that the hybrids are sterile. In this connection I should like to point out the possibility that the elimination of intermediate unit steps between such species as these may be due not only to the instability of certain combinations (since they would split in their offspring), but to the sterility of certain combinations; or, in other words, their inability to produce any offspring. There is evidence, which I need not detail here, to show that sterility in crosses is a condition which may originate relatively suddenly in connection with a series of mutations. In other words, sterility has probably not arisen gradually as the species became farther differentiated, but certain forms are doomed to be sterile with certain other forms

[9] FERNALD, M. L., Rhodora **8**:70. 1906; **9**:106. 1907.

from the moment of their origin, just as certain chemicals will react with each other while others will not.

It seems highly improbable that the specific differences between *S. amplexifolius* and *S. roseus* are directly of selective value to their possessors. We are beginning to learn that natural selection must often act in more roundabout ways, through sterility, etc., and not directly as arbitrator between the possessors of one or other of a pair of differential characters. The character differences themselves must often be innocuous as regards the economy of the plant.

Maianthemum dilatatum (Wood) Nelson and Macbride[10] and M. canadense Desf.

The genus *Maianthemum* has been variously considered as having one, two, or three species. *M. bifolia* DC. in Europe and *M. canadense* Desf. in North America are now regarded as distinct, and a giant form variously known as var. *kamtschaticum* Gmel. and var. *dilatatum* Wood has been attached to the former species. It is clear that *M. bifolium* and *M. canadense* are distinct, and there seems no doubt that this "variety" should be recognized as a third species. Nuttall first recognized it as such. It is found in Western America, from California northward to Alaska, and apparently in adjacent Asia.

This species is essentially a giant *M. canadense* except that the leaves have nearly the peculiar shape of *M. bifolium*. In view of our knowledge of the relation between *Oenothera Lamarckiana* and *O. gigas*, it would not be at all surprising if this also proved to be tetraploid. It is to be hoped that some one will make a cytological comparison of these two species. Their differences are shown in fig. 6. *M. dilatatum* is not only stouter, with larger inflorescence and larger leaves, but the leaves also differ in shape, being broader and with conspicuous basal lobes. This is not at all incompatible with tetraploidy, as we know from the case of *Oenothera gigas*, in which the leaves are very much broader and obtuse pointed.

[10] *Maianthemum dilatatum* (Wood) Nelson and Macbride, Bot. Gaz. **61**:30. 1916. *Maianthemum bifolium* var. *dilatatum* Wood, Proc. Acad. Phila. 1868: 174; *Smilacina dilatata* Nutt. ex Baker, Jour. Linn. Soc. **14**:563. 1875; *Convallaria bifolia* var. *kamtschaticum* Gmel. Cham. and Schlecht. Linnaea **6**:587. 1831.

In the examination of a considerable amount of herbarium material, I have seen no intermediates between *M. dilatatum* and *M. canadense* or *M. bifolium*, although even if such occur it by no means diminishes the possibility that *M. dilatatum* originated from *M. bifolium* or *M. canadense* through a single mutation, for when *O. gigas* crosses with *O. Lamarckiana*, intermediate hybrids are

FIG. 6.—*Maianthemum canadense* Desf. (on the left) and *M. dilatatum* (Wood) Nelson and Macbride (on the right; a portion of one leaf was accidentally broken in taking the photograph).

produced, and these again when crossed back with either parent species produce new intermediate stages. *O. gigas* is also very variable in foliage, probably as a result of the tetraploid condition. If these two classes of variants were found in a population of typical wild *O. gigas* and *O. Lamarckiana*, they would prove very confusing from the systematic point of view, but from the genetic

standpoint their occurrence in no way obscures the relationship of the two species; and the same is true of all other tetraploid species when they come to be known as such.

There seems to be a tacit recognition of *M. dilatatum* as peculiarly related to one of the other species, for it has been classed as a variety, although it is easily as distinct from either *M. bifolium* or *M. canadense* as these are from each other.

If this supposition with regard to this pair of species of *Maianthemum* proves to be correct, then their relationship to each other is very different from that found in our pairs of *Clintonia* or of *Streptopus*, for in the latter cases we found it necessary to assume that pairs had arisen through several divergent steps, accompanied no doubt by free intercrossing of the various forms produced by these successive mutations. In *Maianthemum*, however, we assume one species to have given rise directly to the other, and any intermediates to have arisen later through crossing.

The specimens of *M. dilatatum* examined show little variation in foliage, and this may perhaps be taken as an indication that the species is not tetraploid, although in any case its cells may be expected to be conspicuously larger than in *M. bifolium* or *M. canadense*.[11] Since its leaf shape agrees with that of *M. bifolium*, it is probably best considered as a giant of that species, although it agrees with *M. canadense* in being glabrous. *M. dilatatum* thus bears features of both the other species, although it is entirely distinct from either. We may reasonably assume that it originated from a glabrous variety of *M. bifolium*, which had itself arisen from the type through a negative mutation in loss of pubescence.

Ranunculus abortivus L. and R. allegheniensis Britt.

My attention was directed to this pair of species by Dr. J. M. Greenman. *R. abortivus* has much the wider range, occurring from Labrador and Nova Scotia to Manitoba, and south to Florida, Arkansas, and Colorado; while *R. allegheniensis*, a segregate

[11] Unless, perchance, it is not a cell giant at all. The well known frequency with which Pacific coast species are conspicuously larger than their more eastern congeners makes one doubt the possibility that they are all cell giants. Their greater vigor may result perhaps from an effect of climate. Only a cytological examination can determine these matters.

described by BRITTON, has been found only from Vermont, eastern Massachusetts, and New York to the mountains of North Carolina. The relative distribution of these species is similar to that of the species of *Clintonia* previously considered, although in this case *R. allegheniensis* occurs wholly within the range of the other species. The specific differences may be tabulated as follows:

R. abortivus Linn.	*R. allegheniensis* Britton
Stem leaves divided into oblong or linear, somewhat cuneate lobes	Stem leaves divided into linear acute segments
	Stem glaucous
Petals pale yellow, shorter than the small reflexed calyx	Petals pale yellow, minute
Styles very short, curved	Styles subulate, hooked, nearly half as long as the achene

The main distinguishing feature of these two species is the conspicuous recurved beaks of the achenes in *R. allegheniensis*. The other differences are very inconspicuous and in themselves scarcely noticeable. It seems reasonable to suppose that this species has arisen from *R. abortivus* through a single positive mutation. The idea that these conspicuous beaks might have been gradually developed through natural selection might have been readily accepted at the end of the last century, but has since lost its plausibility. All the facts, both of characters and distribution, are more reasonably explained on the mutation hypothesis. An increased length of beak is, so far as I know, of no considerable use to the plant, although it is possible that the large hook might aid the seeds in transportation by attachment to animals. In distribution, however, the plant, while locally abundant, is restricted in area, and *R. abortivus* surrounds it on all sides except where they both reach the Atlantic coast. This points, not to its having an advantage over *R. abortivus* in the struggle for existence, but more probably to its having originated from that species relatively recently through a mutation, and having since propagated itself and spread with no conspicuous advantage or disadvantage in competition with the parent form.

The wide northerly distribution of *R. abortivus* makes it appear probable that it is the older species and has given rise to *R. alle-*

gheniensis. Only if the distributions were reversed would it appear probable that *R. allegheniensis* had given rise to *R. abortivus*, through a negative mutation in the nearly complete loss of the beak.

Actaea alba (L.) Mill. and A. rubra (Ait.) Willd.

In the genus *Actaea* the species are all remarkably similar in foliage and habit, almost the only sharply contrasting characters being found in the thickness of the pedicels and the color of the berries. All the American forms were formerly treated as varieties of the European *A. spicata* L., but it has become customary to treat them as species. *A. alba* and *A. rubra* constitute a conspicuous pair of these species in eastern North America. They have both been considered varieties of *A. spicata*, but are no doubt worthy of specific recognition. We may first compare them, and then we shall find it profitable to examine the whole genus *Actaea*.

Actaea alba (L.) Mill. (fig. 7)	*Actaea rubra* (Ait.) Willd. (fig. 8)
Leaflets generally more incised, teeth and lobes acute or acuminate	Leaflets ovate, or the terminal one ovate, toothed or somewhat cleft, the teeth mainly rounded or mucronate, or acutish
Raceme oblong	Raceme ovoid
Petals truncate, slender, like transformed stamens	Petals rhombic-spatulate, much shorter than the stamens
Fruiting pedicels thick	Fruiting pedicels slender
Berries ellipsoid (globular-ovoid, Gray's *Manual*), white often with a purplish spot at the end	Berries oval (ovoid-ellipsoid, Gray's *Manual*), red
Flowering a week or two later	
More common westward and southwestward	
N.S. and Anticosti to Ga., west to Minn. and La.	N.S. to N.J. and Pa., west to S.Dak. and Neb.; also Idaho

A. rubra varies in foliage from forms scarcely if at all distinguishable from the typical *A. spicata*, to forms having larger, coarsely

serrate, and less pointed leaves; but the European *A. spicata* L. shows a very similar series of variations, so it is very doubtful if

FIG. 7.—*Actaea alba* (L.) Mill.

there is any constant distinction between these species in foliage. The same must be said of *A. rubra* and *A. alba*. The conspicuous

differences between these species are two: (1) the berries red or white, (2) the pedicels in fruit slender or stout. The latter differ-

FIG. 8.—*Actaea rubra* (Ait.) Willd.

ence is clearly shown in figs. 7 and 8, which also show the great similarity in foliage.

As we shall see, several of the species may produce either white- or red-berried individuals, while the pedicels remain slender, so

that the color of the fruits and the thickness of the pedicels are independent pairs of unit characters, and we may consider *A. alba* as perhaps having originated from *A. rubra* through two mutations, in one of which the chief change was in the color of the berry, while in the other it was in the thickening of the pedicels. If there are two Mendelian pairs here, however, it is difficult to see why the two combination types, (1) white berries and thin pedicels and (2) red berries and thick pedicels, are not of more frequent occurrence. Crossing experiments, if they could be carried out, would doubtless throw light on the situation and would be of very great interest. Red berries perhaps would be dominant over white berries, but since a red tip remains to some at least of the white berries, the white may be dominant and the plants with red-tipped berries heterozygous. Whether there would be any dominance of slender or thick pedicels is impossible to say.

A more careful analysis, however, discloses other differences besides those mentioned. Thus LLOYD[12] describes the differences between the fruits of *A. rubra* and *A. alba* as follows: *A. alba* has its fruit (1) on thickened pedicels, (2) smaller, (3) with a larger tip, (4) with a much thicker "integument," (5) without pulp, (6) with larger and fewer seeds (6 instead of about 12), whose sides are more slanting and their surface smooth (not roughened). They also cite observations of Mrs. STOWELL, who found that in *A. alba* the pedicels were much harder, firmer, and darker, and with much larger starch grains.[13] These authors cite the occasional occurrence

[12] LLOYD, J. U. and C. G., Drugs and medicines of North America. 1884–1885 (p. 232).

[13] Hand sections of the pedicels from dried material of these two species show that she failed to note the essential differences. The ring of wood is the same in structure and diameter in both cases, but in *A. alba* the pith fills the center, while in *A. rubra* it contains an irregular cavity frequently extending as a slit across the diameter of the xylem ring. The increase in thickness of the pedicel in *A. alba* is due to the much greater thickness of the cortical tissue, which is composed of about 7 rows of enormously larger cells than in *A. rubra*. In the latter not only are these cells very much smaller, but the number of rows of cells is only 3 or 4. No differences in the size or shape of the starch grains in the two species were observed, but the large cells in the cortex of *A. alba* contain a great amount of starch, while the small cells of *A. rubra* contain very little. With these structural differences, it might be supposed that the pedicels of *A. alba* would be less firm and rigid than those of *A. rubra*. This point should receive further study.

of plants having white berries on slender pedicels or red berries on thick pedicels, and give it as their opinion that such forms are sports and not hybrids.

MERRIAM[14] has also made observations on the differences between these two species. He found that *A. rubra* has very delicate green pedicels, three-quarters of an inch in length, and berries a half larger than *A. alba*, the pedicels being *hollow*, so that they are easily crushed between thumb and finger. In *A. alba* the pedicels are very thick, red, half an inch in length, the berries small (one-third inch), the pedicels being *solid* or nearly so and not easily crushed. Sometimes the berries in *A. alba* are red, the difference in color being the only change. This suggests that the white of *A. alba* may be dominant to red.

Earlier observations on these species were made by BIGELOW,[15] who described *A. alba* independently under the same name,[16] not knowing that MILLER had described it previously.[17] He points out several other distinctions between *A. rubra* and *A. alba* in his descriptions, which may be summarized as follows:

A. alba Bigelow	*A. rubra* Willd.
Stems and leaves somewhat larger and smoother	
Raceme oblong, twice the length and half the breadth of *A. rubra*	Raceme hemispherical or half ovate
Pubescence of peduncles and pedicels more sparse than usually occurs in *A. rubra*	Peduncles round, smooth, slightly pubescent at top
Pedicels shorter and thicker	Pedicels pubescent, largest at the extremities
Sepals 4, oblong, white, concave, caducous	Sepals 4, oblong, green, striate, concave, caducous
Petals 4–8, white, oval, dilated upward, truncated, deciduous	Petals often 8 or 10, white, oval, acute unguiculate, deciduous
Filaments as long as the petals	Filaments nearly twice as long as petals

[14] MERRIAM, J. S., Bull. Torr. Bot. Club **3**:43. 1872.

[15] BIGELOW, JACOB, Fl. Boston. 2d ed. 1824 (p. 211).

[16] In EATON, AMOS, Manual of Botany. 3d ed. 1824 (p. 155).

[17] Also RAFINESQUE, C. S., Amer. Monthly Mag. **2**:266. 1818.

A. alba Bigelow	*A. rubra* Willd.
Berries milk white, tipped with red, smaller, about 8-seeded, on short, red, incrassated pedicels as large as the common peduncle	Berries shining, cherry red, about 16-seeded, on long filiform pedicels, one-fourth as large as the common peduncle
Flowers a week or two later	

To the differences mentioned on p. 193 we may add therefore (1) greater pubescence of the raceme in *A. rubra*, and (2) filaments nearly twice as long as in *A. alba*. Differences which I have not verified are (3) berries of *A. rubra* with about twice as many seeds, (4) petals more numerous, and (5) sepals green instead of white.

From these facts it is clear that the differences between *A. rubra* and *A. alba* are numerous and affect the fundamental structure of the plant. It becomes a question whether all of these differences could be determined by only two mutations, and this is a matter on which only breeding experiments can throw any light. Of course, it is possible that the quantitative decrease in the pubescence of *A. alba* may be correlated with the increase in thickness of the pedicels, both being structural expressions of the same inner germinal change. Similarly, it is possible, although perhaps scarcely probable, that such changes as smaller size of berries, lack of pulp, larger and fewer seeds, and truncate petals in *A. alba* are all aspects of the same change which made the berries white. The minor differences in shape of berries and leaves have also to be taken into consideration if they are not mere fluctuations.

The frequent occurrence of such vanishing distinctions as those just mentioned affords one of the main difficulties of taxonomic work, and the presence of the "residua" of characters in superposing one species upon another has been thought to offer serious difficulties in explaining the origin of one species directly from another. A careful examination of known mutations, however, shows that similar conditions occur here. Thus, in *Oenothera brevistylis* the main distinctions from *O. Lamarckiana* are in the very short styles and sepal tips and the misshapen stigmas. But minor differences of a quantititave sort are found throughout the plant, notably in the more obtuse tips to the leaves, a feature which shows quantitative variation in the foliage of each individual.

Hence the occurrence of such minor differences in addition to the conspicuous ones is not a difficulty which requires to be explained by the assumption often tacitly made, namely that inherited environmental effects have led to these slight divergences.

We may now examine briefly the whole genus *Actaea* as it stands at present. Taxonomically considered, the species are as follows:

1. ACTAEA ALBA (L.) Mill.

Actaea spicata var. *alba* L. Sp. Pl. 504. 1753.
Actaea alba Mill. Gard. Dist. ed. 8. 1768; Icon. Corn. Canad. *t.* 77.
Actaea americana var. *alba* Pursh, Fl. Am. Sept. **1**:366. 1814.
Actaea brachypetala var. *alba* DC. Reg. Veg. **1**:385. 1818.
Actaea brachypetala var. *microcarpa*[18] DC. Reg. Veg. **1**:385. 1818.
Actaea pachypoda Ell. Sketch **2**:15. 1824.
Actaea alba Bigelow, Fl. Bost. ed. 2. 211. 1824.
Christophoriana americana Park. Theatr. Bot. 379. 1640.
Christophoriana americana, racemosa, baccis niveis et rubris Morison, Hist. Univ. Oxon. 8. *s.1. t.2. f.7.* 1680.

2. ACTAEA RUBRA (Ait.) Willd.

Actaea spicata var. *rubra* Ait. Hort. Kew **2**:221. 1789.
Actaea rubra Willd. Enum. Hort. Berol. 561. 1809.
Actaea longipes Spach, Hist. Vég. Phan. **7**:388. 1839.
Actaea brachypetala var. *rubra* DC. Reg. Veg. **1**:385. 1818.
Actaea americana β *rubra* Pursh, Fl. Am. Sept. **2**:367. 1814.

Said by GREENE[19] to differ from the European *A. spicata* L. in that (1) the lowest leaf is inserted high above ground (not radical), and (2) the berries are cherry red, not black. As noted by PURSH and embodied in the name of DECANDOLLE, the American species also has shorter petals, the petals of *A. spicata* L. being as long as the stamens. According to SPACH, however, they are sometimes shorter.

A variety of the European *A. spicata* under the names *A. erythrocarpa* Fisch. and *A. rubra* Ledeb. differs in having red instead of black berries, presumably a simple unit change.

2*a*. ACTAEA RUBRA DISSECTA Britton, having decompound leaves and incised leaflets, has been recorded from Lincoln County, Ontario, in BRITTON and BROWN, Ill. Fl. **2**: 55. 1897.

[18] "Baccis parvis albis subrubellis, pediculis incrassatis." This is apparently the pink-berried form sometimes mentioned.

[19] Pittonia **2**:108. 1890.

FIG. 9.—*Actaea rubra* var. *gigantea* Gates

2*b*. ACTAEA RUBRA var. **gigantea,** n. var. (fig. 9).—A forma typica differt, grandior omnibus partibus; caule crasso, 3–4 mm. in diametro; foliolo terminale 10–13 cm. longo, latitudine maximo 6–12 cm., grossē dentato, dentibus ad basin 1 cm. aut plus diametro; subtus rugosē-venoso; pedicellis gracilibus sed quam iis typi crassioribus, 14–20 mm. longis; baccae rubrae, 7–14 mm. longae.

This striking giant variety is represented by several specimens and is very distinct, being much larger and coarser in all its parts. It is not improbably a sporadic tetraploid mutation, and it is to be hoped that some one will examine the size of its cells and the number of its chromosomes in comparison with *A. rubra* when the opportunity offers. To be quite consistent, this should be recognized as a species, but there is some advantage at present in regarding it as a variety of *A. rubra* and so indicating its obvious relationship. It forms with *A. rubra* a "pair of species" (cf. figs. 7 and 8). Of the specimens cited later, one (DODGE, 1896) is considerably smaller in all its parts, and perhaps represents an intermediate hybrid between *gigantea* and *typica*, such as we should expect to find where the forms intercross.

The length of the type specimen of *gigantea* from the base of the peduncle to the tip of the central leaflet is about 28.5 cm., while the corresponding length in typical specimens of *rubra* is about 17.5 cm.

Specimens: *J. Fowler*, Fredericton, New Brunswick, July 20, 1892, Herb. Mo. Bot. Gard., *type; E. L. Sturtevant*, Framingham, Massachusetts, July 8, 1890; *Pammel* and *Ball* 236, Ames, Iowa, August 1896; *Chas. K. Dodge*, near Port Huron, Michigan, August 2, 1896 (in part).

A cultivated specimen from Halle in Herb. Mo. Bot. Gard. indicates that the European *A. spicata* L. also probably has a variety *gigantea*.

Hand sections of the pedicels of *A. rubra* were compared with var. *gigantea*. A series of 21 measurements of cortical cells gave in the former case an average diameter of 7.6 μ. A similar series from var. *gigantea* gave an average of 9.4 μ, from which it would appear that the cells are larger, although the difference is not a conspicuous one.

3. ACTAEA NEGLECTA Gillman, in Lloyd, Drugs and medicines of N.Amer. 235. 1884–1885.

Actaea rubra forma *neglecta* Robinson, Rhodora 10:66. 1908.

The distinctions of this form from *A. alba* are stated by GILLMAN as follows: *pedicels green and slender*, leaves 4-ternately compound, racemes ovate, peduncles longer, berry (white) larger, seeds few (about 4) and much rougher, very slightly grooved. By contrast *A. alba* is said to have thick red pedicels, leaves 3-ternately compound, racemes oblong, peduncles much shorter, berries smaller, seeds more numerous (5–7) and nearly smooth, with deep grooves.

4. ACTAEA ARGUTA Nutt. in Torr. and Gray, Fl. N.Amer. **1**:35. 1838.

Actaea spicata var. *arguta* Torr. Pacif. R.R. Rep. **4**:63. 1856.
Actaea rubra var. *arguta* Greene, Pittonia **2**:108. 1890.
Actaea californica Greene, Ottawa Nat. **16**:36. 1902.

This western species differs from *A. rubra* chiefly in (1) being larger and stouter, (2) having spherical berries; the leaves are also less divided. Its distribution is from British Columbia to Montana, Idaho, the Black Hills of South Dakota, New Mexico, and California.

A. californica Greene is stated to be very distinct from *A. arguta* Nutt. in its "rhombic ovate acute petals (commonly 3 or 4), its peculiarly broad and almost obtuse leaflets, which are also not much incised," stems often several from the same rootstock as in *A. viridiflora* Greene. The description is inadequate to determine the characters.

So far as can be judged from specimens, *A. arguta* is not so large or stout as *A. rubra* var. *gigantea*.

4*a*. ACTAEA ARGUTA var. EBURNEA Ckll. in Daniels, Fl. Boulder, Colorado. 119. 1911.

Actaea eburnea Rydb. Mem. N.Y. Bot. Gard. **1**:153. 1900.

A. eburnea was described by RYDBERG from Montana. It closely resembles *A. rubra* in size and form of fruit (ellipsoid, 9–12 mm.×6 mm.) and the form of petals, but the berries are perfectly white, the plant taller, leaflets broader and more acuminate, and the teeth sharper. It is nearer to *A. arguta* in habit, but differs in color and size of fruit and somewhat in the form of the petals. The berries are about 12-seeded, the seeds obliquely pear-shaped, triangular with a rounded back. Its distribution is given as Idaho and Utah to the Black Hills of South Dakota, and also on Mount Mackay, Ontario, and Willoughby Mountains, Vermont. The relationships of this form require further study.

4*b*. ACTAEA ARGUTA var. ALABASTRINA Lunell, Am. Midland Nat. **2**:123. 1911. North Dakota.

This variety has berries spherical or subspherical, 8–10 mm. in diameter, differing from *A. arguta* only in color. Apparently it occurs sporadically. It is possible that *A. eburnea* with ellipsoidal berries should be classed with *A. neglecta*. It differs from var. *alabastrina* in the shape of the berries.

4*c*. ACTAEA ARGUTA var. **pauciflora,** n. var.—A forma typica decedit, foliorum supra rarē et minutissimē pilosa; inflorescentia 3–4-florā, bracteis obsoletis, petalis duo ellipticis, paulatim in unguiculam abientibus.

Plant large, leaflets 6.5 cm. long, 4–5.5 cm. broad, ovate to oblong, often obscurely 3-lobed, acuminate, rather coarsely incised-dentate, not caudate, upper surface sprinkled with minute shining hairs as in *A. caudata*, lower surface almost completely glabrous except for sparse minute hairs along the main veins; inflorescence composed of only 3 or 4 flowers on short slender pedicels 3–5 mm. long, bracts very small and inconspicuous; petals 2 on the flowers observed, blade elliptic, passing gradually into a claw of nearly equal length reaching nearly to the ends of the filaments; stamens 3–6 mm. in length; pedicels and upper part of peduncle fine pubescent; berries unknown.

Type specimen: *Trelease* and *Saunders*, Harriman Alaska Expedition 3785, Juneau, Alaska, June 8, 1899, Herb. Mo. Bot. Gard.

5. ACTAEA VIRIDIFLORA Greene, Pittonia **2**: 108. 1809.

Described from open rocky places, Arizona, in flower July 10, 1889. Since collected in New Mexico (*O. B. Metcalfe* 305, 372) and in southern Colorado (*Baker, Earle*, and *Tracy* 235). The latter, however, has longer and stouter pedicels[20] and is evidently a different thing. The following specimen is also referred to this species: *C. F. Baker* 681, Black Canyon, Colorado, 1901.

This species appears to be well characterized by the following features: (1) stems a cluster from a clump of roots; (2) flowering very late, leaves less developed at time of flowering; (3) racemes reaching 5–6 inches long, particularly narrow, elongated and dense; (4) pedicels all of equal length, shorter (6–13 mm.); and (5) "remarkably short greenish stamens." The petals are said to be "rather numerous," ovate to nearly lanceolate, usually acutish, little shorter than the stamens.

A. viridiflora also occurs in two varieties having respectively red and white berries, but apparently differing in no other particular. The latter might be known as *A. viridiflora* var. *alabastrina*. The white variety presumably originates through a mutation, being found interspersed with the red.

5*a*. ACTAEA VIRIDIFLORA var. **Clementiorum,** n. var.—A forma typica differt, folioliis angustioribus, saepe ad basim cuneatibus; inflorescentiâ perbreve et floribus paucioribus; stamina flavis.

This variety would be recognized as a separate species if it were sharply marked off from the type of *A. viridiflora*, but in all characters, except perhaps the color of the stamens, there is a gradual transition series. In the extreme form of the variety the leaves are much divided, and both the terminal and lateral leaflets are for the most part cuneate at base, the teeth serrate. The terminal leaflet is 5–6 cm. in length and 12–30 mm. in width at the widest part.

[20] The flowering pedicels are longer and not so stout as in *A. alba*. All other known forms have slender pedicels.

The inflorescence is short (15–35 mm.) and narrow, pedicels short (5–10 mm.) and slender, densely pubescent in anthesis. The petals number about 4, very broadly ovate, sharply narrowed to a claw of equal length; stamens yellow, short but exceeding the stamens. These differences may be summarized as follows: (1) leaves highly decompound, the leaf segments narrower, often cuneate at base, (2) raceme short, containing fewer flowers, (3) stamens yellow.

Specimens: *F. E.* and *E. S. Clements* 239, Jack Brook, Colorado, June 20, 1901 (two sheets; fruit red), Herb. Mo. Bot. Gard., *type; C. F. Baker* 318, near Pagosa Peak, Colorado, August 1899, fruit white.

A photograph of the Jack Brook Station by CLEMENTS shows a dense group of the plants, so that several stems probably arise from one rootstock, as in *A. viridiflora*. The racemes in this group vary considerably in length and most of them bear white berries, but in a few the berries are red. Evidently there is free intercrossing of the type and the variety in Colorado, with blending in foliage and length of raceme, while the red and white berries form a sharply alternating character.

6. ACTAEA CAUDATA Greene, Ottawa Nat. **16**: 35. 1902.

Described from Chilliwack Valley, British Columbia (*J. M. Macoun* 33550 in part). This species is insufficiently known and needs further study. *A. caudata* seems to be chiefly characterized by (1) young petioles and leaflets minutely villous, the latter along the veins beneath; (2) upper face sprinkled with minute, rigid shining appressed hairs; (3) leaflets with a long lance-linear perfectly entire acumination; (4) petals 2 or more, two-thirds the length of the stamens, blade elliptic with a flattened claw of the same length. The berries are unknown.

Specimen: *Shaw*, Selkirk Flora, 279. 1904.

7. ACTAEA ASPLENIFOLIA Greene, Ottawa Nat. **16**: 35. 1902.

Described from Yakutat Bay, Alaska (*Funston* 14, 1892), and another specimen collected in Alaska by *A. W. Gorman*. It agrees closely with *A. caudata* in the pubescence of leaves and stems, the caudate tips to the leaflets,[21] and the presence of usually two petals. The main distinguishing features are (1) leaflets deltoid-lanceolate, incisely lobed to a greater degree than in other species, the lobes serrate; (2) raceme very short and few-flowered; (3) petals less than half the length of stamens, blade round-obovate or almost orbicular, claw equally short.

Specimens: *Trelease* and *Saunders*, Harrison Alaska Expedition 3786, Yakutat Bay, Alaska, June 20, 1899; *E. C. Smith*, Seattle, Washington, April 23, 1889; *Mrs. Moore*, Montana, 1894;[22] *Frank H. Lamb* 1353, Baldy

[21] This feature is apparently not constant.

[22] This specimen has a larger inflorescence than the type, and the leaf tips are not caudate.

Peak, Chehalis County, Washington, 3500 ft. elevation, July 24, 1897;[23] *G. E. Coghill* 151, Pecos River, T.R., New Mexico, August 5, 1898;[24] *Fendler* 12, New Mexico, 1847.[24]

It seems obvious that in studying the species of such a genus as *Actaea* the variations which lead from one species to another have, at least in many cases, not been gradual or continuous, but definite and in certain well defined directions. It seems clear that the nature of these variations has been determined by the internal structure of the germ plasm, the environment acting for the most part merely as a releasing stimulus.

Spiraea tomentosa L. and S. alba DuRoi

Spiraea alba DuRoi[25] (fig. 11) is better known under the name of the European species *S. salicifolia* L. It has a diagonal distribution across North America from North Carolina, New York, and Ontario to Saskatchewan, Iowa, and Missouri, and is also found in Siberia. The members of this pair of species are evidently much less closely related than in the other pairs I have mentioned. Fossil predecessors of *S. tomentosa* (fig. 10) show that the tomentose group of species has long been separated from the non-tomentose group. In other words, the characteristic tomentum on the ventral leaf surfaces appeared in these forms long ago, and heredity has handed it down since that time. It is only the accident of distribution, therefore, that makes *S. tomentosa* and *S. alba* a pair. Indeed, we could better consider the group a trio, for in its more eastern range in the Atlantic states and eastern Canada *S. tomentosa* is paired with *S. latifolia* (Ait.) Borkh. (fig. 12). The latter species is still frequently known under the name *S. salicifolia*. Certain more recent segregates from *S. salicifolia*, such as *S. corymbosa* Raf. and *S. virginiana* Britton, which are more restricted in their distribution, also occupy portions of the range of *S. tomentosa*.

[23] The foliage is transitional to *A. arguta*, but the inflorescence and pubescence agree with *A. asplenifolia*.

[24] Resembles *A. asplenifolia* in foliage, but has less pubescence along the veins.

[25] *Spiraea alba* DuRoi = *S. salicifolia* var. *alba* Ehr. *S. alba* is distinguished from the European *S. salicifolia* (1) slightly in leaf shape, (2) the inflorescence is a conic not a narrow panicle, (3) the sepals are triangular not ovate, (4) the petals are not pink and are more nearly orbicular.

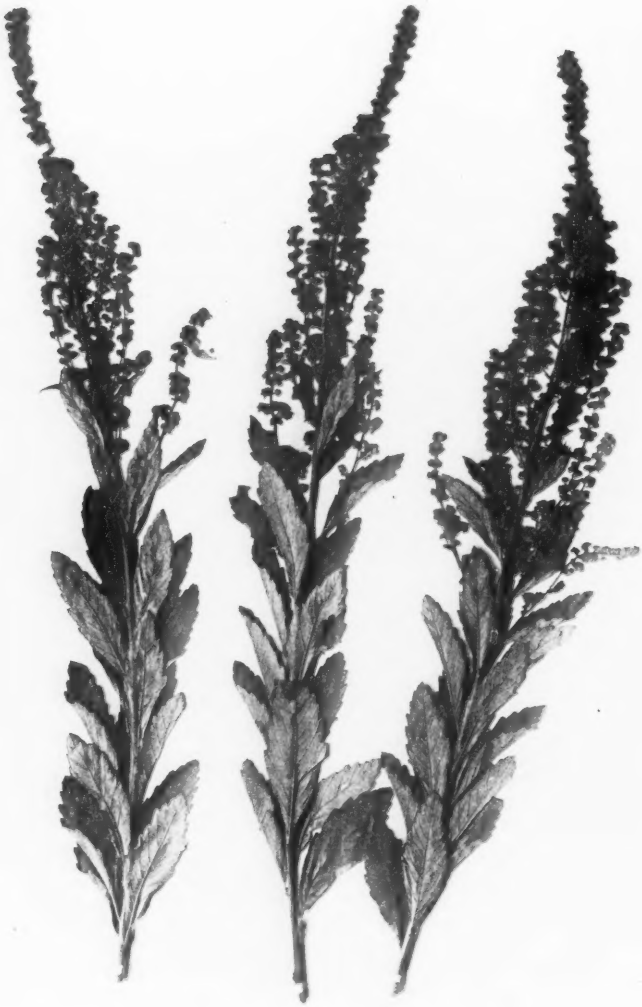

FIG. 10.—*Spiraea tomentosa* L. var. *rosea* (Raf.) Fernald

FIG. 11.—*Spiraea alba* DuRoi

The range of *S. latifolia* is stated to be from Newfoundland to Saskatchewan, western Pennsylvania, and Virginia. Judging from specimens, the Newfoundland form is probably distinct. *S. tomentosa* occurs from Nova Scotia to Manitoba and south to Arkansas and Georgia. The eastern portion of its range, therefore, is covered by *S. latifolia* and the more western part by *S. alba*.

The present tomentose group is represented by *S. tomentosa*, *S. Douglasii* Hook., and *S. dasyantha* Bge.[26] *S. Douglasii* occurs in western America from British Columbia to California. It differs from *S. tomentosa* chiefly in (1) leaves slightly different in shape and serrate only above the middle; (2) tomentum on ventral leaf surfaces always white, never rusty; (3) follicles glabrous, not divergent. *S. dasyantha* occurs in China and Japan. The fossil species *S. Andersoni* Heer, from Alaska, is considered most nearly related to *S. tomentosa*.[27] A somewhat variable condition of *S. tomentosa* has been segregated as var. *rosea*.[28] It differs from the type in having a less compact inflorescence, and the follicles, though tomentose, are not lanate, becoming glabrate as they mature. The type is generally confined to the coastal plain and the Atlantic states, while this variety is found farther south and west in Wisconsin, West Virginia, and North and South Carolina. The variety merges gradually into the type of the species and the two features, (1) degree of compactness of inflorescence and (2) amount of pubescence on the follicles, appear to vary independently.

S. tomentosa and *S. alba* must then be looked upon as a spurious pair, while either *S. tomentosa* and *S. Douglasii* or *S. alba* and *S. latifolia* constitute real pairs. The tomentose character itself not improbably originated by a step, although it may have increased in amount later. Since *S. tomentosa* and *S. latifolia*

[26] RYDBERG (Fl. N.Amer. **22**:251. 1908) has described two other "species," *S. tomentosula* from Washington and *S. subcanescens* from South Carolina, both of which are considered to be probably hybrids, the former of *S. Douglasii* and *S. lucida* Dougl., the latter of *S. tomentosa* and *S. alba*.

[27] See MAXIMOWICZ, C. J., Adnotationes de Spiraeaceis. Acta Horti Petropol. **6**:105–261. 1879.

[28] FERNALD, M. L., The inland variety of *Spiraea tomentosa*. Rhodora **14**:188–190. 1912. *S. tomentosa* L. var. *rosea* (Raf.) Fernald; Fluk. Alm. 393. *pl. 321. fig. 5*; RAFINESQUE, New Flora **3**:62. 1836.

FIG. 12.—*Spiraea latifolia* (Ait.) Borkh.

occupy much the same habitat, it can scarcely be supposed that the tomentum is a character which determines survival, although of course it is conceivable that a change in its physiology renders necessary this extra protection. Possibly experiments in removing the tomentum from young leaves, if it could be done without injury, might answer this question.

Before leaving this genus I wish to point out a condition in another species of *Spiraea* which can only be supposed to have originated suddenly through a mutation. It is very difficult to conceive a gradual and continuous transition from the foliage of such species of *Spiraea* as we have been considering to that of *S. millefolia* Torr., now known as *Chamaebatiaria millefolium* (Torr.) Maxim., which occurs from Idaho to Arizona and southern California. In this species and the related *C. glutinosa* described by RYDBERG[29] from Nevada, the leaves are pinnately divided and the primary divisions are again divided, as in many ferns. Various other features separate this genus from *Spiraea* proper, but the finely bipinnate type of leaf must have been derived from leaves which were nearly entire, and it is easiest to conceive this as having occurred in a few well marked steps. Complete continuity in such a process is out of the question.

Summary and conclusions

In this paper, which is an attempt to apply the concepts of mutation to the practical discrimination of species and the understanding of their relationships, I have selected for consideration several pairs of species and their relatives. It is found that these pairs bear very different relationships to each other, both as regards their characters and their distribution. They may occupy the same territory or adjacent areas, they may overlap, or be widely separated. Again, one species may be a giant of the other, or may differ by a few sharp differences which have probably originated as units, or may show differences which cannot be externally analyzed in this way.

Thus, *Spiranthes cernua* is a tetraploid giant of *S. gracilis* or a related species. *Maianthemum dilatatum* is perhaps a cell giant

[29] Fl. N.Amer. **22**:258. 1908.

of *M. bifolium;* and *Actaea rubra* var. *gigantea* is probably a cell giant of *A. rubra*, from which it has apparently arisen by a mutation. In the case of *Clintonia borealis* and *C. umbellulata*, the peculiarities of the latter probably represent a differential of three or four definite and independent variations. In this way would arise a series of forms, all of which have been extinguished except the two remaining. This hypothesis differs from the Darwinian theory of natural selection only in assuming that the inherited variations are usually not infinitesimal, but bold and definite strokes. We are merely applying the conceptions gained from the facts of experimental breeding.

The pair *Streptopus amplexifolius* and *S. roseus* presents a similar problem. There are four main pairs of character differences between these species. They may be assumed to have arisen through a series of mutations from a common ancestor. Intercrossing would lead to various combinations and in some cases blends of these mutant characters. Many such combinations would be gradually eliminated through their own instability or their sterility in producing offspring, leaving finally the present pair of species as survivors. The differences between *Ranunculus abortivus* and *R. allegheniensis* are such that the latter, which is more limited in its distribution, may be reasonably assumed to have arisen from the former through a single positive mutation. It is very difficult, if not impossible, to believe that the conspicuous beak of the achene, which is the main peculiarity of *R. allegheniensis*, could have been developed gradually through natural selection. It is much more probable that the character has no selective value and is merely inherited because it has appeared as a germinal variation.

In *Actaea*, after a somewhat detailed analysis of the differences between *A. rubra* and *A. alba*, it was found desirable to consider the whole genus as it now stands, and incidentally three new varieties were described. The differences between *A. rubra* and *A. alba* are much more numerous than might have been anticipated, yet two mutations are perhaps sufficient to account for the origin of the latter from the former. The thickening of the pedicels in *A. alba* was found to be due to the fact that the rows of cortical cells are

more numerous and the cells themselves enormously larger. The minor differences, as shape of berries and leaves, in which the distinctions are of the vanishing order, are not at variance with the mutation hypothesis, for they are also found when known mutations are compared with their parent forms; for example, in *Oenothera rubrinervis* the foliage characters are not sharply differentiated from those of *O. Lamarckiana*, but are quantitatively separated.

Finally, *Spiraea tomentosa* and *S. alba* constitute a spurious pair of species. In reality *S. tomentosa* is paired with *S. alba* in one part of its distribution and with *S. latifolia* in another part; but *S. tomentosa* has itself been derived from a tomentose ancestor represented by a fossil form from Alaska. Hence its relation to the other two species is more remote, and it only forms a pair with either of them through the accident of their present distribution.

It seems clear that the mutation conception can be applied with advantage to the consideration of all such species relationships, but, of course, crossing experiments and cytological investigations provide the only final answer to the specific questions involved, and it is to be hoped that such investigations will be undertaken, at least in some of the genera discussed in this paper.

The photographs which illustrate this paper were kindly taken by Mr. C. H. THOMPSON. They are all from specimens in the herbarium of the Missouri Botanical Garden, and all specimens cited in this paper are from the same source. I am indebted to the Director, Dr. GEORGE T. MOORE, for the facilities provided for making these observations, and to Dr. J. M. GREENMAN for much kindly help in connection with the work in the herbarium.

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ABSCISSION IN MIRABILIS JALAPA

FRANCIS E. LLOYD

(WITH PLATE XIII AND TWO FIGURES)

The purpose of this investigation

Although the phenomenon of abscission has received no small amount of attention, a study of the literature fails to discover any real uniformity of opinion as to the precise steps involved. This divergence I have already indicated in an earlier paper.[1] As I shall take occasion at a future time to attempt a general critique of the whole subject, my present purpose is a restricted one, namely, the consideration of a more recently published account of abscission in the common "four o'clock" (*Mirabilis jalapa*), by E. HANNIG.[2] My curiosity touching the matter, so far as this plant is concerned, was aroused by HANNIG's statement that the mode of abscission, which is common only to *Mirabilis* and *Oxybaphus*, does not accord with any previously observed accounts, and is made by him, therefore, to represent a new type, while his figures appeared to me not to support his contention. It seemed to me rather that a more exhaustive study of even the published drawings of LOEWI[3] and of TISON[4] alone would have led HANNIG to see a strong resemblance between those and his own figures. Before material of *Mirabilis* was available for my own observation, I ventured therefore to believe that he was not justified[5] in formulating a new type of abscission. Since then I have obtained an abundance of material, and after a careful study of the process in question in both stems and leaves, I find myself unable to alter my opinion.

[1] Abscission. Ottawa Nat. **28**:41–52; 61–75. 1914.

[2] Untersuchungen über das Abstossen von Blüten, etc. Zeitschr. Bot. **5**:417. 1913.

[3] Blattablösung und verwandte Erscheinungen. Proc. Vienna Acad. **1**:166–983. 1907.

[4] Recherches sur la chute des feuilles. Mém. Soc. Linn. Normandie **20**:125, 1900.

[5] LLOYD, *loc. cit.*, p. 72.

The purpose of this paper is to support a challenge of the correctness of HANNIG'S views and of the evidence brought to support them, and to substitute evidence to show that abscission in *Mirabilis* does not represent a new type. To do this will entail a detailed account of the process as understood by me, and to compare this with that of HANNIG as exhaustively as possible.

Digest of Hannig's view

HANNIG recognizes in general two methods of abscission of flowers (that is, of the supporting axis), namely, (*a*) by the solution of the middle lamella of the cells of the abscission zone; and (*b*) by the complete dissolution of an entire layer of cells. The latter constitutes the new type in question, and is, according to HANNIG, "of a very peculiar sort." His account runs as follows:

Two or three cell layers of the abscission zone, which is 12–20 cells thick, are destroyed and go completely over into solution. This destruction proceeds first by the thinning of the membranes of the affected cells. These membranes become more strongly refringent, while the cell contents take on a granular character. The process is first recognized by the absence of intercellular spaces, followed by granular degeneration of the cell, ending finally in the liquidation of the whole layer of tissue. The process begins at a particular point under the epidermis, which is soon broken. The tear then extends around the whole cortex and finally inwardly into the pith. The vascular bundles appear to be broken across mechanically. The persistence of starch in the abscission cells, while the surrounding tissues, except the endodermis, lose it, is asserted, but even those cells which are reduced to extremely thin membranes, simply because of their too rapid destruction, also retain it. Starch in the abscission cells is therefore of no particular significance. HANNIG was unable to find any evidence, by means of suitable reagents, of chemical alteration of the cell walls to distinguish them from those of neighboring cells. From these conclusions the further one is drawn that the entire cells of the abscission layer, without recognizable previous alteration of the cell membranes, go into solution (p. 430).

He further holds that there is present in all plants which shed their leaves in laboratory air a preformed primary abscission zone ("Trennungsschicht") which is more or less sharply set off from the neighboring tissues. It is pronounced in some species (*Salvia*, *Fuchsia*, *Impatiens*, etc.), but less so in *Begonia* and *Mirabilis*. This zone is to be seen also at the base of the internode. It is the less marked the older and thicker the internode, but still can be recognized, since the cells are smaller and display new transverse cell walls. In *Impatiens*, however, these new walls are so infrequent that the abscission zone is recognizable thereby with difficulty. The manner of separation is the same in leaf, stem, and flower (pedicel, peduncle).

The larger nodes offering the best opportunities for observation, HANNIG further says that it is not the entire abscission zone which is dissolved, but a layer ("Lösungsschicht") of a few cells which are not distinguishable beforehand in any way. This dissolved layer is placed usually in the middle, but sometimes toward the base of the abscission zone, and without reference to the direction of the cell layers. Contrasting *Mirabilis* with forms like *Impatiens* (in which the cells of the abscission zone are set free by the dissolution of the middle lamella), it is pointed out that, while in these the abscission surfaces exposed after rupture are granular or pulverulent in appearance, due to loosened cells, those in *Mirabilis* are mucilaginous. In this plant then the abscission zone is only a more or less broad band of tissue, in the approximate middle of which the separation layer ("Lösungsschicht"), itself not in any way differentiated as to its cells, occurs. This zone is conceived by HANNIG only as a zone of tissue capable of choristic response, to adopt FITTING'S term.

The present account of abscission in Mirabilis

The preceding account, being a digest of HANNIG'S statement, has been set out with some fulness and detail in order to obviate the possibility of a criticism which may be in any way superficial or gratuitous. HANNIG'S resulting contention that in *Mirabilis* (and in the allied *Oxybaphus*, which I have not examined), a type of abscission obtains which is "quite new and distinct," is

presently examined in the light of the facts as they are now to be presented.

THE ABSCISSION ZONE

While it is known that abscission in the internode occurs, in general, near its base, it is impossible to connect it with visible structural peculiarities, preformed specificially with respect to the process. HANNIG says as much in admitting that, although frequently or usually delimited by the smallness of the cells or the occurrence of transverse divisions, these marks are not always to be discovered.

In very young internodes there is nothing at all to enable one beforehand to fix upon the tier (usually one only) or tiers of cells which become involved later. In older structures the configuration of the cells for a short distance *both above and below* the node is practically identical, and, although transverse walls are plentiful in the prosenchyma, one cannot regard these as having any more definite relation to abscission than the still older walls. Just as abscission sets in one can frequently recognize the evidence of a renewal of cellular activity in a zone of several tiers of cells, for in these may occur many new, very thin transverse walls ("secondary meristem" of earlier authors). Abscission finally intervenes usually at the upper limit of this zone (pl. fig. 4), and not in the middle or below, although several tiers of cells may finally take part. There is no other structural zone which deserves designation in this connection. Since, however, even such a zone as this occurs only sometimes in older organs, in which a general rejuvenescence then appears to be demanded before abscission can supervene, it can hardly enable us to delimit or define a reactive zone. This zone of rejuvenescence is the "Folgermeristem" of VON MOHL, in which his "Trennungsschicht" arises, and, as WIESNER[6] has pointed out, the latter does not always arise in a secondary meristem. KUBART[7] took the trouble to point out the proper use of these terms, and there appears to be no good reason to change their usage.

[6] Über Frostlaubfall, etc. Ber. Deutsch. Bot. Gesells. **23**:49. 1905.

[7] Die organischen Ablösung der Korollen, etc. Sitz. Akad. Wiss. Wien. **115**: 1491. 1906.

Evidence, however, will be furnished to show that a zone of tissue, here designated the abscission zone, composed of a number (in large internodes about 10–12) of tiers of cells *below* the separation layer, but not above, shows enough visible alteration, quite aside from cell divisions and distinguishable by optical and chemical means, to warrant the conclusion that it constitutes a physiologically active zone concerned in abscission. It is not simply a secondary meristem, if by this term is implied a renewal of transverse cell division, since this does not always occur, but a mass of cells going through the initial steps, consisting most obviously of chemical alteration of the cell wall, leading to abscission, and of which only some cells finally conclude the process. These latter, which are sufficiently active to go far toward and many of them finally to conclude separation, constitute the separation layer. A secondary meristem may or may not intervene.

THE CENTERS OF ABSCISSION ACTIVITY AND THE DIRECTION OF ITS PROPAGATION

According to HANNIG, abscission begins under the epidermis (which soon tears) and travels around the cortex, finally passing through the vascular bundles and into the pith.

The fact appears to be that the first step in abscission (separation) in internodes takes place in the innermost cells of the cortex at two points in a plane normal to the plane of the opposed leaves. From these points the process is propagated outwardly, inwardly, and around the stem, but more rapidly toward the center of the pith than toward the epidermis. Indeed, separation is usually completed in the pith before the epidermis is ruptured (pl. figs. 2, 4). Because of the longitudinal growth of the separation cells, there is a considerable displacement (0.1–0.2 mm.) of the parts to be separated, and there is a synchronous rupture of the passive portions of the xylem. We therefore find the vascular tissues to be in an advanced state of disruption (text fig. 2*d*; pl. fig. 14) before the epidermis is ready to break. When this finally occurs, the fracture of the separation layer is most easily accomplished.

In young stems and leaves the same procedure seems to be followed, although, because of the delicacy of the organs and the rapidity with which abscission is consummated, it is more difficult to follow. There is undoubtedly, however, a great deal of irregularity. Instances have been seen in which the epidermis was fractured and the wound gaped open, in the manner suggested by HANNIG'S fig. 9, before the separation was completed in the pith.

CHEMICAL ALTERATION IN THE WALLS OF THE ABSCISSION CELLS

Proof that separation is preceded by a chemical alteration in the cell wall is the following.

(1) If a section (preferably a fairly thick one) is treated with strong KI/I, the walls of the abscission cells will appear to have an indefinite pale greenish hue. If the reagent is washed away gradually, as the yellow of the iodine disappears, the walls show a pale blue, which fades away as the washing is prolonged. An open diaphragm is necessary. This color reaction[8] is much more pronounced if the section is first boiled for a minute in weak (4 per cent) HCl, which, because of a previous alteration of the cell walls, attacks them but does not affect the remainder. Not merely the cells actually involved in separation, but *all the cells below the separation plane for a distance of 0.5 mm.* (in a large node) show the blue reaction, but in less and less degree the farther from the separation plane. Above this the transition is sudden, only a portion of the adjacent cell walls of the cells immediately above being altered.

(2) In earlier stages of abscission, however, the blue reaction is not visible, but an indication of chemical alteration is to be seen in the failure as compared with the adjoining, unaltered cells, to color strongly with iodine.

(3) The staining capacity of the wall of the separation cells is obviously reduced. They will not hold Bismarck brown (pl. fig. 9), so that they become almost colorless, while the unaltered

[8] The reaction was observed under circumstances of evident physiological significance by GREEN (The soluble ferments and fermentation, p. 97) and by myself (Development and nutrition of the embryo . . . . in the date). Ann. Rep. Mo. Bot. Gard. 21:103. 1910.

walls remain deeply stained. The relation is the same to ruthenium red, but not so markedly.[9] One must distinguish between the staining capacity of the entire wall of the abscission cell before elongation sets in, and that of the delicate membranes which continue to invest the protoplast during and at the culmination of elongation. The latter seem to stain readily, at least more so than the former.

(4) The optical quality of the walls is clearly altered, since, with the iris diaphragm at a given opening, a much more narrowly outlined object picture is obtained than in the case of an unaltered cell. HANNIG states that this condition obtains (pp. 428–429).

(5) There is a readily appreciable amount of swelling of the cell walls, most marked in those which are thicker. Consequently, the thick-walled cells of the collenchyma and prosenchyma sheath are most favorable for observation. This phase appears to be passed through quite quickly. Transverse sections display this condition most abundantly (text figs. 1, *a*, *b;* 2, *a*, *b*, *c;* pl. figs. 5, 6, 12, 13).

(6) Following this the walls become altered to such an extent that, when successfully stained with ruthenium red, the substance of the wall appears as a flocculated mass, separated from the protoplasm by a delicate membrane (pl. figs. 6, 13). Whether the flocculation is due to the preservation in alcohol or to the action of ruthenium red[10] matters less than the fact that the walls are in a condition either of flocculation or in one which allows it. When in this condition the walls frequently show breaks of such character that they can be explained only on the supposition that it is in the condition of a gel. As abscission nears completion, this granular matter is much reduced in amount, apparently by hydrolysis. The product may be absorbed by the abscission cells, for which it may very well be regarded as a source of energy. It is not superfluous to insist that this granular matter is not cytoplasm, although it may easily be mistaken for it, especially as displacements of the

[9] Successful differential staining is obtained best by means of quite dilute solutions.

[10] I have noticed that ruthenium red flocculates the pectic (?) mucilages derived from ripe fruits of *Diospyros*, but have been unable to see any flocculation in fresh, unstained material of *Mirabilis*.

delicate cell walls confuse the microscopic picture. That the cells whose walls are thus being changed are not degenerating, witness the fact that cell divisions may be taking place, and the condition of the nucleus and cytoplasm.

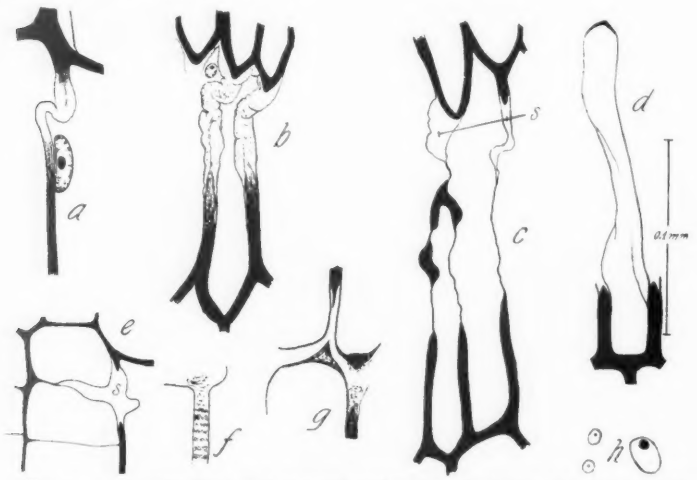

FIG. 1.—*a*, Longitudinal wall of a cell undergoing abscission, showing softening in a restricted zone, near which the nucleus lies (cf. pl. fig. 9); *b*, more advanced condition, in which the walls are very much altered, but remain bounded by a delicate tertiary membrane (cf. pl. figs. 5, 6, 12, 13); *c*, condition of extreme extenuation of the tertiary membranes, seen separated in the upper part of the figure; portion of unaltered wall remains suspended in these membranes (cf. pl. fig. 10, a portion of which was selected for this figure; also pl. fig. 11); *d*, cell after separation, the tertiary membrane intact and evidently disarticulated at its upper end from two more distal cells (cf. pl. fig. 8); *e*, partially separated pith cells, the tertiary membranes remaining intact; *f*, the flocculated remains of a hydrolyzed membrane held between, and broken up by, the extending tertiary membranes in the pith; this condition may be seen in preparations represented by pl. fig. 7; *g*, mutual disarticulation of the ends of adjacent cells; *h*, small nuclei from parenchyma cells near the abscission layer, but not taking part in abscission; and a large one from an abscission cell in an advanced stage; drawn to identical scale.

THE SEPARATION LAYER; ITS COMPOSITION

Only one tier of cells may be involved in the act of separation (pl. figs. 5, 7), this usually in smaller, younger leaves and internodes, except that in young leaves there is a tendency to increase the number of layers involved. In larger nodes, in which there

is considerable thickening of the walls of certain tissues (especially the prosenchyma sheath and cortex), from one to four or five tiers of cells (pl. figs. 3, 4, 15) may undergo some of the changes in form leading to separation, although it is seldom that the cells of more than one tier actually complete the process. This is usually the uppermost, although occasionally one below this may conclude separation, but in a restricted region only. In no case, however, do all the superimposed separation tiers pass entirely across the stem, for in the pith usually one tier only is engaged, only occasionally more. The maximum number of tiers activated is to be

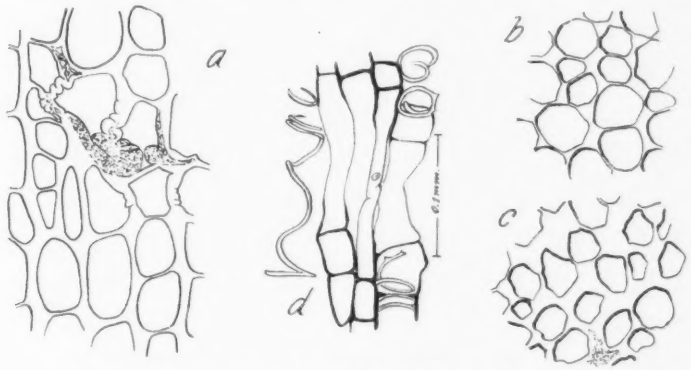

FIG. 2.—*a*, Transverse section through a portion of the prosenchyma sheath, showing swollen walls; *b*, *c*, transverse sections through cortex; in *b* the walls are unaltered, in *c* they are swollen, but still incompletely hydrolyzed; *d*, longitudinal view of a few wood parenchyma cells ready for separation, with adjacent wood vessels fractured (cf. pl. fig. 14).

found in the prosenchyma sheath, where the resistance against longitudinal growth is greatest, due to the thickness of the cell walls and to the presence of the vascular tissues.

The mechanical resistances doubtless affect also the direction taken by the separation layer. Although, broadly speaking, this follows the direction of the tiers of cells, it tends to depart from it, and, as the epidermis is approached, to pass into the cell tiers below (pl. figs. 3, 4), as if the tendency to lie normal to the abscission cell axes was to some extent overcome by the tendency to lie normal to the stem axis. The maximum irregularities in direction

occur where there is the greatest heterogeneity of structure or maximum mechanical resistance, namely, in the oldest stems and in the vascular tissues, so markedly indeed that I have frequently observed in some plants an entire misdirection of the plane of abscission, in that it comes to lie parallel with the vascular bundles for some distance. Conversely, the greatest regularity is seen in the pith and medullary rays, where, however, in spite of the homogeneity of the tissues, minor local departures from the ideal path may be observed (pl. figs. 1–4).

CYTOLOGICAL BEHAVIOR OF ABSCISSION CELLS

a) THE RÔLE OF STARCH.—Previous to abscission, there is an abundance of starch in the general region below the separation layer, while it is withdrawn from above it except from the starch sheath. As abscission progresses, the starch disappears below, from the middle of the pith last. From this point it has disappeared usually by the time abscission is well advanced throughout its whole extent. In the early stages of abscission starch is to be found within all the separation cells themselves, but it largely or entirely disappears as abscission is completed. The place where the absence of starch is first to be noted is in the prosenchyma sheath next to the starch sheath. The latter also loses starch in the vicinity of the abscission tissues. When abscission is complete, I have observed in some cases various but always small amounts of starch, a few large grains in the middle of the pith and a very few scattered grains elsewhere or none. The fact therefore appears to be that, as abscission progresses, the starch in the abscission cells decreases until it is very materially reduced in amount or entirely disappears. The inference is that it is used by them as a source of energy, expended in the active growth leading to separation.

HANNIG described the abscission layer as being marked by a transverse band of starch grains, but adds that those cells which are reduced to extremely thin membranes and are more or less collapsed still contain starch. He suggests that this is due to the rapid autolysis of these cells; but the purpose of this essay is to

show that such autolysis does not occur, and, if this be true, HANNIG's inference is not justified. The appearance of collapse or compression is accidental, and that the abscission cells in *Mirabilis* should appear so is not to be wondered at, in view of the facts presented in this paper. While some starch grains may indeed persist even until after abscission is completed, the granules are quite obviously very much reduced in size, so that, in a particular plastid, the stroma enveloping the granules may readily be seen between them, and the granules of starch may appear as minute points.

To draw a sure inference from the presence of starch in, or its absence from, separation cells is at best precarious. For example, the separation cells in *Hydrangea*, in which "maceration" occurs as in *Impatiens*, retain their starch; while the immediately neighboring cells lose it. But, when abscission is complete, the separation cells, while entirely loosened from each other, are quite alive. In such a case as this the starch may not be used at all, and may be retained because the loosening of the cells prevents its movement (this being essentially HANNIG's explanation), or it may have been secreted in amount much in excess of that needed. But it is, in any event, doubtful if the loosening of the cells would prevent the movement of starch.

b) THE BEHAVIOR OF THE CELL WALL.—Evidence has already been brought forward to show that, previous to any changes in the dimensions of the cells involved in abscission, there occurs an alteration of the cell walls. The degree of alteration appears not to be the same throughout the whole of the cell wall, judging by its behavior during those steps preceding separation. For the sake of simplicity, I take an ideal case, that of a fairly thick-walled cell of the prosenchyma sheath, in which the physiological activity proceeds in a plane normal to the axis of the cell (pl. fig. 15), it being premised that this plane may lie in any oblique direction (pl. fig. 10) passing through any of the walls. The changes observable are presented seriatim.

(1) The cell wall is softened in the previously described manner, most completely, however, in a narrow zone nearer the upper

end of the cell and therefore nearer the plane of final separation, in the event that this supervenes (text fig. 1, *a*, *b*).

(2) The cell grows longitudinally, the maximum extension of the walls occurring where the maximum softening has taken place, and concurrently with it. The length of the cell may increase four or fivefold. The total length of the longest cells in the prosenchyma sheath at the time of separation has been found to be 0.2 mm. or slightly more (text fig. 1, *c*, *d*).

The protoplasmic utricle also becomes greatly extenuated during this period of growth and the cytoplasmic membrane is most delicate. The nucleus is disposed in the transverse zone of elongation, usually increases in size, and remains perfectly normal in appearance until the completion of separation and even still later (text fig. 1, *h*). It may lie against the wall or be suspended in or near the axis of the cell. Occasionally a very delicate transverse wall is laid down just previous to or at the time of elongation. Transverse walls may have been formed still earlier, but the fact that the cellular activity may take this form during the process leading to separation must be taken as evidence of vigor rather than of degeneration. Further, it is not possible to suppose that this behavior is consonant with a loss of turgor, supposed by HANNIG to occur, while the disappearance of the starch suggests a constant accession of solutes for maintaining turgor.

FURTHER ANALYSIS OF THE CHANGES WHICH OCCUR IN THE CELL WALL

It is of prime importance, in view of the purpose of this paper, to analyze fully the changes undergone by the walls which display elongation. These are more readily appreciable in the thickest of them. The wall shows first of all evidence of chemical alteration resulting in a physical change which allows it to be drawn out.[11] There is no evidence that the middle lamella alone is altered, but rather does it appear that the whole wall, excepting only a delicate membrane limiting the lumen of the cell, is softened.

[11] For a review of various accounts of the way in which the cellulose membrane is affected by enzymes, see JONES, L. R., Pectinase, etc. N.Y. Agric. Exp. Sta. Techn. Bull. 11. Nov. 1909.

probably by hydrolysis (text fig. 1). This remaining membrane[12] it is which continues, during elongation of the cell, to invest the protoplasm, and which, because of the disappearance of the remainder of the wall, comes into intimate contact with those of neighboring cells (text fig. 1, *c*). Being very soft and delicate membranes, on coming into contact with each other they cling together and appear as a single membrane, which is optically scarcely resolvable as double, so that its composition must be argued from the occasional separation of its components (pl. fig. 11), and from their conjoint greater thickness. These changes are for obvious reasons, more prolonged where the walls, or portions of the walls, are most thickened, as in the collenchyma and prosenchyma sheath, and are therefore more readily seen in such tissues (pl. figs. 5, 6, 12, 13).

Not only are these thin membranes proper to neighboring cells separate from each other, but they become separated also at their upper ends (in some cases at their lower ends) from the thick membrane of the cells in the next tier above, only the lower ends of which are chemically altered, but enough for this. The iodine reaction demonstrates this to be the case. That this separation of walls actually obtains can be proved by taking rather thick sections of suitable material and, after a slight treatment with 5 per cent sodium hydrate, or weak hydrochloric acid,[13] pulling the portions separated by the abscission layer apart on the slide, when the separation cells pull away in many cases without breaking. Investing their free ends one usually finds a thicker portion of wall (pl. fig. 8) evidently derived from the chemically altered but unstretched membranes with which the ends of the abscission cells were in contact (text. fig. 1, *d*, *g*). It is more difficult to

[12] These membranes investing the protoplasts of the abscission cells were seen by TISON. His sketches, however, do not convey an accurate conception of their appearance, nor does he seem to have followed their genesis closely; for example, he recognizes no growth. LOEWI, on the other hand, saw the elongation of the walls, without following the changes in the walls themselves, or comprehending the nature of the thin walls consequent on these changes.

[13] One is not compelled to use these or any reagents, but they facilitate the obtaining of particularly fine preparations. Since the treatment is not sufficient to hydrolyze even the thinnest of the membranes, it cannot be objected to.

demonstrate that these membranes are free by means of untreated sections, although not at all impossible.

The result of all this is to produce a separation cell, the ends of which are invested by thicker, physically relatively unaltered walls (text fig. 1, *d*) with a transverse zone between them, narrow at first, but becoming quite wide at length, of extremely thin membrane. Viewed *en face*, the thicker portions of the wall show their shallow pits, well seen in the prosenchyma sheath, and between them and the thin portions the pits are seen to disappear and the membrane itself to show the granulation or flocculation due to the chemical alteration (pl. fig. 15). If, as has been premised, the thinning out has proceeded in irregular, more or less oblique zones, very various topographic conditions (pl. fig. 10) ensue without any violation of principle.

Although the above view of the nature of the thin walls seems to accord entirely with the facts, it remains true nevertheless that they could be accounted for by supposing that, as the old walls become softened and broken down, entirely new thin walls are laid down by the growing protoplasm. This is TISON'S view. While it is altogether possible, or even probable, that some new wall material is being laid down, the optical evidence is against the idea that the wall is entirely new. One can, if with some difficulty, resolve a relatively unaltered membrane in contact with the protoplast in the portions of the cell which are not elongated, and can determine its continuity with the thin membranes in the zone of elongation. The continuity comes out clearly when the chemical alteration of the rest of the wall is far enough advanced so that the optical differences presented by the primary and secondary membranes on the one hand and the tertiary membrane on the other are obvious (pl. figs. 6, 13; text fig. 1, *b*, *f*).

The process as described is the same for all living elements, for example, cambium cells and wood parenchyma. The xylem vessels are fragmented in one or more transverse planes, according to the number of tiers of cells involved in abscission. Tyloses in various degrees of development and in various numbers are to be seen both above and below the abscission plane, but, as SWART[14] has shown experimentally, they cannot effectually hinder the

[14] SWART, N., Die Stoffwanderung in ablebenden Blaettern. Jena. 1914.

passage of water, since they are not sufficient either in size or numbers, and are not present at all in many vessels. The formation of tyloses appears to be rather a wound response, more or less incomplete at the time of rupture of the leaf, since the greater development, if incomplete, is found below the abscission plane, that is, the tyloses appear in the general region and at the same time as the general wound response.

The younger phloem elements, those quite near the cambium cells and with difficulty distinguishable from them, appear to behave the same as the latter. The older, on the other hand, which show a development of callus (though whether this is synchronous with the development of tyloses, as TISON holds, I cannot at present say) show indications that they behave in a quite passive manner. One can detect no definite zone of thinning in the wall, but, if stretched at all, they act merely as a soft yielding material, becoming thin throughout their whole length, quite as a soft india-rubber band behaves, and finally break. The very small size of the elements makes it difficult to be quite sure of this conclusion, but I have seen no evidence which would lead to any other.

The abscission cells of the epidermis behave as do the parenchyma cells, except for a certain asymmetry due to the unyielding cuticle, which merely breaks at last, after being loosened by alteration of the adjacent cellulose membrane.

The final condition of the active abscission cells is then as follows. The walls are locally, and it may be very irregularly, much extended and extremely thin. The thin membrane of each cell is distinct from that of any neighboring cell, and also from the proximal end walls of the cells above. The cytoplasm consists of a correspondingly delicate membrane, but shows no alteration in the direction of degeneration. The same is equally true of the nucleus, which is in appearance quite the same as evidently normal nuclei elsewhere, but that it is frequently much larger. When this condition has been reached, abscission may be regarded as complete. After the cuticle is broken, the abscission cells near by quickly collapse, while the shrinkage of the parts above, due to curtailment of water caused by local evaporation and by the severance of the vascular elements, produces a shearing and rupture of the abscission cells. At a slight touch, the whole layer of weakened

cell wall cells gives way, partly by pulling away the entire thinned walls (pl. figs. 5, 8) and partly by tearing them. The resulting wound surfaces of the separated parts seem, both to sight and touch, mucilaginous, as HANNIG observes, but this is not due to the entire dissolution of abscission cells, but to the fact that, on account of the thinness of the walls, many of them break, allowing their mucilaginous contents, accompanied by protoplasm, to ooze out on to the exposed wound surface. The contrast, in this respect, with the analogous surfaces of other plants (notably for example, in *Parthenium* and *Impatiens*) cannot be adduced as evidence of the nature of the process leading up to it. The only thing we might say of it is that it would lead one to examine the antecedent facts more closely, in order to detect essential differences, should they obtain. In this case I believe there are none.

Summary

1. Previous to abscission activity proper there is no antecedent structural indication of the position of the abscission zone. In young organs usually only one tier of cells is involved, while in old ones (internodes) evidence of physiological activity is to be seen in 10–12 tiers (the *abscission zone*) approximately. The greatest activity is to be seen in 1–5 tiers of cells, constituting the *separation layer*, at the upper limit of the abscission zone. Here new transverse walls occur in varying numbers, giving rise to the "Folgemeristem" of VON MOHL. The parenchyma at the base of a mature internode shows many transverse walls, which, however, have nothing to do with abscission, and do not constitute the criterion of an abscission zone. HANNIG'S "Lösungsschicht" and the "separation layer" of this paper may be regarded as identical, while his "Trennungsschicht" does not coincide with the "abscission zone" as here conceived.

2. Abscission begins in the internode near its base at two points which lie in a plane normal to that of the opposed leaves, and in the innermost part of the cortex. From these two points it is propagated outwardly toward the epidermis, and inwardly toward and into the pith. When more than one tier of cells is engaged, the changes which overtake them usually progress most rapidly in the uppermost (most distal) tier.

3. The walls of all the cells of the abscission zone, as defined in this paper, are altered chemically during abscission. The greatest degree of alteration takes place in the cells which are actually involved in separation (namely, those of the separation layer), in which the alteration, by hydrolysis, proceeds so far as to procure the complete digestion of a part of the primary and secondary walls, thus allowing a great extenuation of the tertiary walls, their separation from each other, and from the only partially altered primary and secondary walls of the next distal tier of cells.

4. The separation layer is composed of one tier of cells only in very young organs, and from 1 to 5 in older. Of these tiers, usually only the uppermost proceeds to complete separation. Mechanical resistances, which are greater in older parts, appear to influence the direction of the plane of separation.

5. The behavior of starch in the separation layer and adjoining tissues during abscission indicates that it is a source of energy for the separation cells during their growth.

6. The final separation results from the digestion of portions of the primary and secondary cell walls of the separation cells, leaving the protoplasts invested by a tertiary membrane which grows independently of adjoining membranes, from which it is quite free.

7. Neither the cytoplasm, nor the nuclei, nor any part thereof, displays degeneration changes. On the contrary, cytoplasm, nuclei, and nucleoli bear evidence of greater physiological activity, and are quite alive and normal when separation is achieved. There is meanwhile no loss of turgor.

Conclusion

From the foregoing facts it is concluded that abscission in *Mirabilis* is not procured by a separation resulting from the complete solution and destruction of a layer of tissue, as held by HANNIG, and does not therefore constitute a new type of abscission. Contrariwise, the mode of abscission accords wholly, as to all essential details, with that which has been shown to occur in such forms as *Gossypium*, *Aristolochia*, etc.

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EXPLANATION OF PLATE XIII

FIGS. 1–4.—Longitudinal sections through the bases of internodes, displaying variations in the regularity of form and number of separation layers; in fig. 1, a single tier of cells, in fig. 2, two tiers for a limited distance, in fig. 3, three tiers, and in fig. 4, four or even five tiers of cells are involved.

FIG. 5.—A small portion of the separation layer in the cortex in which abscission is complete; the abscission cells are more or less distorted, but those in the middle of the figure but little, and in these the normal nuclei can be seen; 3 cloudy masses can here be seen, and these are shown on a larger scale in fig. 6.

FIG. 6.—Longitudinal walls in a swelled and partly hydrolyzed condition; they can be seen to alternate with the protoplasts in position; ruthenium red; compare with figs. 11–13.

FIG. 7.—Abscission cells in the pith, when separation is readily possible; on the left of the central cell, the altered cell wall has broken, the tertiary membranes alone remaining intact; the protoplasm and nucleus are clearly normal.

FIG. 8.—The intact membranes of abscission cells remaining after separation has been procured; this preparation was secured by pulling apart a section such as that in fig. 5; at the free ends of the cells the membranes are seen to be somewhat thicker (cf. text fig. 1, *d*).

FIG. 9.—Cortical tissue in a very early stage of abscission, showing the reduced staining capacity of the walls of the cells proceeding toward abscission; Bismarck brown; there is no reduction in the thickness of these walls at this time.

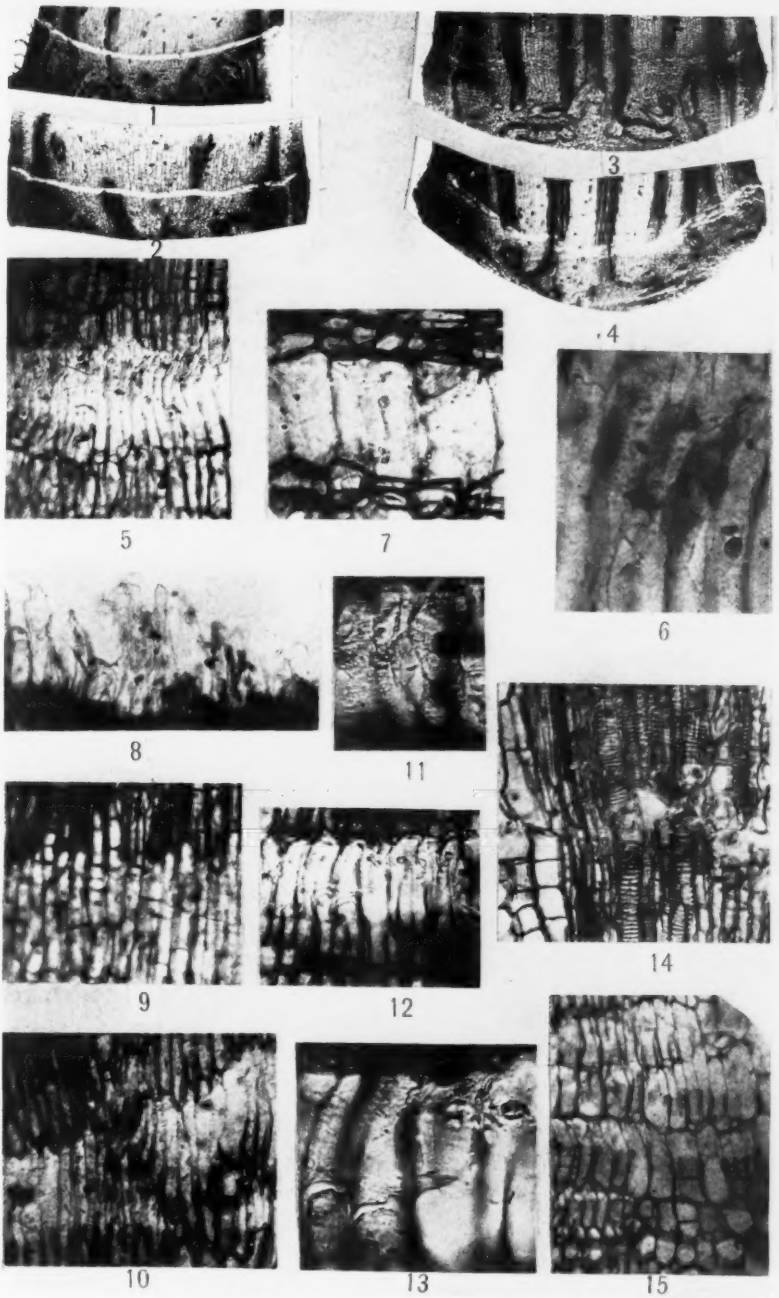
FIG. 10.—A more advanced condition, in which the walls are thin and drawn out; there is further shown the transfer of the abscission plane from one cell tier to another; small, relatively unaltered portions of cell walls are seen suspended by the thin membranes.

FIG. 11.—Between the two protoplasts may be seen an oval intercellular space inclosed by two delicate tertiary membranes; these have been set free by the complete hydrolyzation of the remainder of the wall, of which nothing more is to be seen.

FIG. 12.—Medullary ray cells in abscission, in which the cell walls are hydrolyzed so far that they now appear granular or flocculated; only the wall in the center of the figure shows this well; this has been further magnified and is shown in fig. 13, in which can be seen the tertiary membrane on the right of the altered cell wall; ruthenium red.

FIG. 14.—The fragmentation of the wood vessels by the wood parenchyma and cambium cells which undergo active abscission; tyloses may be seen.

FIG. 15.—Medullary ray tissue, in which several tiers of cells are in a somewhat advanced stage of abscission; thicker walls show pits *en face;* the great delicacy of the thinned out membranes is well brought out here.



LLOYD on MIRABILIS

THE DEVELOPMENT OF THE CONCEPTACLE IN FUCUS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 212

MABEL LEWIS ROE

(WITH PLATES XIV–XVII)

Introduction

When engaged upon a study of *Splachnidium rugosum*, the writer was forced to work out a new theory for the development of the conceptacle, since methods previously described did not offer sufficient explanation for what was found in *Splachnidium*. It seemed probable that such might also be the case in more advanced members of the Fucaceae, particularly *Fucus*, and it was with this in mind that a re-examination of this well-known form was undertaken. The results have proved interesting enough to warrant the reinvestigation.

Material and methods

For the material the writer is greatly indebted to Professor CHARLES J. CHAMBERLAIN, who had at his disposal paraffin cakes of young tips of *Fucus edentatus* Delapyl. and Kjellman (*F. furcatus* Agardh et al.) and of *Fucus vesiculosus* L., of which he had also mature tips containing ripe conceptacles. In addition, he furnished the writer entire plants of *Splachnidium rugosum* Grev., collected by him at Glen Cairn, near Cape Town, South Africa. To Professor JOHN M. COULTER the writer is indebted for material of *Fucus serratus* L., which was collected at Pictou, N.S., by Mr. J. CRERAR MCDONALD, at the request of Professor W. G. FARLOW. All the material, except that used for fig. 20, was cut 5–8 μ in thickness, parallel to the long axis and at right angles to the flat surface of the thallus. Material for fig. 20 was cut 15 μ thick. The sections were stained with Haidenhain's iron alum haematoxylin and given a light touch before mounting with Grübler's Lichtgrün dissolved in equal parts of absolute alcohol and clove oil. The former stain brought out nuclear and cytoplasmic detail, while the latter gave in sharp relief all cell walls and layers of mucilage.

Previous work

The first attempt at detailed, histological description of the origin of the conceptacle was that of KÜTZING in 1843 (**13**). He speaks there (p. 92) of the appearance on the surface of the thallus of small circular depressions, "Fasergrübchen" (cryptostomata), which have a raised border and contain finely septate ("gegliederte") hairs. He says that in their structure they resemble conceptacles ("Hüllenfrüchte") and seem to have a certain relation to them, although present in certain forms which do not possess conceptacles. Moreover, they appear on sterile as well as on fertile plants. He reports them sometimes lacking on *Fucus vesiculosus*, although usually present. He offers an ingenious explanation of the use of hairpits to the plant, describing a fusion of hairs developing a new plantlet as an adventitious shoot upon the parent.

He calls the conceptacle a closed-over sorus ("eingestülpter Sorus") and says (p. 98) that the layer forming the inner wall of the cavity is nothing other than a slight modification of the superficial layer of the cortex which becomes covered over and bears antheridia and paraphyses ("Samen und Nebenfäden trägt"). This cover conforms in structure to that of the superficial layer ("Rindenschicht") of the thallus.

AGARDH in 1848 (**2**) describes in the Fucoideae (p. 2) superficial cells prolonged into hairs, which project freely beyond the surface of the frond, either scattered or massed in a small bundle; this bundle is bounded by an elevated margin of adjacent cells. Such a body he calls a "cryptostomatum." The conceptacles ("scaphidia") he describes as suspended in the anastomosing filaments in the interior of the thallus, and hollowed out beneath the surface (p. 181). In the same place he mentions the occurrence of hairpits ("cryptae minutae") on sterile portions of the frond which are analagous to conceptacles on fertile parts ("scaphidiis in parte fertili analogae"), but that such "crypts" are often lacking. He describes a thin membrane in the early stage covering the mouth of the cavity which is a portion of the superficial layer ("quae tantum pars strati superficialis est"), but that the mouth of the cavity is later always open ("sed demum eadum semper evadunt ostiolo hiantia").

SACHS in 1875 (**26**) says (p. 227) of the conceptacle of the Fucaceae:

"These conceptacles are not formed in the interior of the tissue, but as depressions in the surface which become walled in by the surrounding tissue, and so overgrown that at length only a narrow channel remains, opening outward. The layer of cells which clothes the hollow is thus a continuation of the external epidermal layer of the thallus, and since the filaments which produce the antheridia and oogonia sprout from these, these latter are, morphologically, trichomes."

In the same year REINKE (**21**) published an account of *Fucus vesiculosus*, followed a year later by a much more inclusive record of investigations (**22**) of a large number of closely related genera. In each paper he discusses the origin and development of both hairpits ("Fasergrübchen") and conceptacles. On p. 337 he gives the details. The first indication of a pit is noticed on the lip close to the growing point, where a few neighboring epidermal cells separate themselves from other tissue (comparable to formation of resin ducts in conifers) and form between them an intercellular space filled with mucilaginous substance. Also the cortical cells, just beneath, are involved in this separation, and the cavity thus developed widens into flask-form, wherein the original meristematic cells take on the usual character of the border cells. These border cells develop first into papillae, and by subsequent division into hairs which project beyond the mouth of the pit.

The conceptacle is regarded by REINKE as a structure comparable to the flower of angiosperms, and is a metamorphosed thallus apex and branches. In this metamorphosis hairpits are changed into conceptacles (p. 338). The details of development are about as in the hairpit (p. 339). On the exterior the cavity is rendered firm by the epidermis and a few layers of cortical tissue. The epidermis stops short at each side of the ostiole; the walls are formed by small, nearly isodiametric cells corresponding to the cortex. Hairs spring from the base of the cavity and project beyond the ostiole. Because of the place of origin (close to the growing point) and similarity in method of development (separation of groups of cells from adjacent external cells, etc.), he considers the conceptacle the homologue of the hairpit (p. 340), and

that up to a certain stage there is no recognizable difference between the two ("auf diesem Stufe giebt es keinen erkennbaren Unterschied zwischen Fasergrübchen und Conceptaculum; wir haben es mit der Erscheinung zu thun dass ein der Anlage nach identisches Organ sich in einem Fall zu vegetativen, im anderen Fall zu reproductiver Thätigkeit entwickelt").

The next important contribution to this subject was the paper by BOWER in 1880 (**6**), whose work has been generally accepted since. In his introduction he gives a very brief résumé of previous work, given in the foregoing in greater detail. He first describes the development of the conceptacle and then compares it with that of the hairpit, which he regards "as an incomplete sexual conceptacle" (p. 37). He agrees with ROSTAFINSKI (**25**) as to behavior of segments of the apical cell, namely, that each segment divides first by a wall parallel to its free surface; the outer cell again divides in two planes at right angles to each other and to the first wall, thus giving four cells which may repeat the same method of segmentation as the original segment. A slight modification of this behavior initiates a conceptacle (or hairpit). Upon completion of a linear series of cells, activity in horizontal division ceases, leaving the terminal ("initial") cell in a depression as surrounding tissue continues active growth. This "initial" first loses its internal tension and later shrinks back against the basal segment beneath. Later segments of this basal portion (cortical in origin) line the bottom of the cavity, whereas the sides are derived from the limiting tissue (external layer) and from subjacent cortical tissue. This is the method for *Fucus*.

In *Himanthalia* (pp. 46, 48), the lining of the cavity is derived entirely from limiting tissue. In *Halidrys* he finds (p. 45) that

> "The basal cell divides at first by walls strongly inclined to one another. The cells thus produced do not at any early stage divide by walls parallel to the surface of the cavity. The result is that the conceptacle usually appears . . . . as though lined by a layer of cells continuous with the limiting layer; but as part, at least, of this tissue is derived from the basal cell, this conclusion is inadmissible. Meanwhile the initial cell (or group of cells) has been completely thrown off by the swelling of the wall dividing it from the basal cell. Later, as in other plants of this group, the cells of the lining tissue put forth papillae which develop further into hairs."

BOWER pays considerable attention to the "central column."

"The change of the substance filling the cavity has advanced so far that the unaltered portion immediately surrounding the remnants of the initial cell forms a central column of irregular outline. This stretches from the basal cell to the neck of the conceptacle, and is connected with the walls of the conceptacle by thin strings, which, like itself, have remained as yet unchanged" (p. 40).

He offers as an explanation that there are three substances present: (*a*) a swollen form of cell wall, which is not true cellulose, but is similar to the central portion of older cell walls of the tissue of the larger tangles; (*b*) a substance coincident with mucilage; and (*c*) a substance akin to cuticle. Later the connecting strings are ruptured.

In conclusion (p. 47), BOWER states that

"In all the cases described, the formation of the conceptacle is preceded by the decay of one or more cells which occupy a central position with regard to the changes which follow. The number of the cells thus removed is various, and the manner of their distribution is not constant. . . . . A point which is not so obvious, but which appears of similar constancy, is that the cell or cells which decay are in all cases members of a linear series. It depends upon the activity of division, in a direction tangential to the surface of the thallus, how this series is characterized; whether, as in *Fucus*, where the division is slow and even ceases, the apical cell of the series hangs behind the surrounding tissue; or whether, as in *Himanthalia*, where the division is often repeated, the series is elongated, and, protruding beyond the surface of the thallus, is called a hair"; and (p. 48): "This variation in activity of tangential division accounts for the want of uniformity in number of the cells thrown off in different species, and even in the same species. . . . . The differences in mode of development (in the early stages at least) depend upon the difference in activity of tangential division of the cells of the central series."

BOWER regards the hairpit as an incomplete conceptacle, but that "The homology of the two structures is so clearly proved that I shall be justified in proposing . . . . the name 'neutral conceptacle' (p. 44). I think it is important to convey at once the relation which appears to exist between them and the true conceptacle."

VALIANTE in 1883 (**29**) describes the method of development for *Cystoseira* and related genera. Primordia develop in the apical grooves of ultimate branches, a hollow being formed just as in

early stages of hairpits. In either case, one or more peripheral cells at some point on the inner surface of the groove lose their power to increase in size and are left in a small cavity by active growth of surrounding cells. The initial (or initials) do not disintegrate, but by transverse divisions grow into hairs which project beyond the mouth of the conceptacle. He says he can find no traces, in his material, of decay of elements preceding formation of a cavity, as described by BOWER for *Fucus*. He finds hairpits developed among the conceptacles at apices of fruiting branches, but that in such cases the cavity is less pronounced than usual. He considers these a convincing proof of the close relationship between hairpits and conceptacles.

OLTMANNS (**19, 20**) considers that the method as described by BOWER is essentially constant for all the Fucaceae, with very unimportant exceptions, namely, that the cells abutting on the "initial" have the chief work in organizing a conceptacle, sharing this with the "basal" cell, whereas the "initial" seldom remains intact, but either disintegrates or else grows into a hair ("überall kommt ihr zweifellos irgend eine nennenswerthe Funktion nicht zu"; **20,** p. 516). OLTMANNS regards the conceptacles and hairpits as homologous structures, but takes exception to BOWER'S conclusion that hairpits are "neutral (sterile) conceptacles," for he believes that the hairpits have become sexual (**19,** p. 82).

BARTON in 1891 (**3**) made the next contribution, in her study of *Turbinaria*. She found the conceptacles and hairpits developed in essentially the same way as described by BOWER, VALIANTE, and OLTMANNS, the "initial" cell being persistent. "The initial cell divides longitudinally, and both cells, after again dividing transversely near the top, grow into hairs, the upper division of the initial forming the swollen base of the mature filament. These filaments fill the fully grown conceptacle" (p. 224).

She objects decidedly to BOWER'S terms "neutral" and "sterile conceptacle" as "conveying an idea of abortive growth" (p. 223). Her view is that the two bodies are of "equal antiquity" and "a later development in the ancestors of the Fucaceae than the reproductive organs." "I consider neither form a development of the other, and the fact that one conceptacle contains reproductive

organs, the other nothing but paraphyses, is an interesting point, but does not bear on the phylogenetic history of the conceptacles themselves." She then proposes the term "vegetative conceptacle" as meaning "those cavities in the thallus which have been developed only in a vegetative direction." In commenting on BARTON'S theory, MURRAY (**18**) says (p. 60) "I know so little about the ancestors of the Fucaceae, that I must be content with a respectful attitude toward this statement."

In their research upon *Splachnidium*, MITCHELL and WHITTING in 1892 (**17**) find that the conceptacle is developed by alteration of one of the epidermal cells, close to the apical cell, which becomes peculiarly modified but is inconsequential; however, they consider it the homologue of BOWER'S initial, although it takes no further part in developing the conceptacle, the real work falling to the neighboring cells.

In interesting connection with work on the conceptacle is that on hairtufts and sori in groups outside the Fucales.

MITCHELL in 1893, in a study of *Hydroclathrus* (**16**), finds groups of hairs analagous to "Fasergrübchen" (cryptostomata) of the Fucaceae. In development they certainly resemble the conceptacles, and her description recalls REINKE'S work. She speaks of an isolated cell or group of cells which become separated from surrounding epidermal tissue. Transverse division results in formation of hairs from these isolated cells. Meanwhile growth of the thallus leaves the isolated portion in a slight depression. Later, the sporangia which are developed in close proximity around the hairpits mature, and after liberation of the spores the sporangial walls disappear and the basal cells originate new growth. Finally, all the sporangia disappear and these hairtufts, with central depressions, are left scattered over the thallus, persisting throughout the life of the plant. MITCHELL concludes that although not a true conceptacle, such as in the Fucaceae, yet the growth of both hairs and reproductive organs is initiated by alteration in form and subsequent division of epidermal cells which might with truth be called "initial" cells. "My observations do not exclude the possibility of the initiative being taken by a small group of initial cells dividing simultaneously, instead of a single one."

MURRAY (**18**) states that the development of both cryptostomata and conceptacles follow the method described by BOWER. He quotes BOWER and then adds "These words appear to me to be the true guide of those who investigate the development of such bodies" (p. 60). In conclusion he states that comparison of the conceptacle of the Fucaceae with cryptostomata either in the heart of a sorus, as in *Adenocystis* and *Hydroclathrus*, or apart from the sorus, as in *Alaria* and *Sacchorhiza*, and with the situation in *Asperococcus*, in Cutleriaceae and in Dictyotaceae "points very significantly to a possible origin of cryptostomata. I anticipate, from further research into the development of these bodies, evidence that may enable us to dispense with the ancestors of the Fucaceae, of which, however, I would speak with respect" (p. 63).

BARTON in 1898, in a study of *Soranthera* (**4**), *Colpomenia*, and *Chnoospora* (**5**), found an interesting development of reproductive bodies in connection with cryptostomata, giving further evidence along the line already noted by MURRAY in his study of *Adenocystis*. In each case a saucer-like depression initiated the central portion of a cryptostoma and later of a sorus with reproductive sacs, whether these latter be plurilocular or unilocular. The important fact is that both portions of the sorus always originate as modifications of the superficial layer of the vegetative body.

HOLTZ in 1903, in his work with *Pelvetia* (**11**), describes a method of development little different in essentials from BOWER's. Several epidermal ("initial"?) cells cut off a series of segments beneath to form a sort of pad of meristematic cells; then the external portions break down more or less, forming a cavity. The inner portions line the base of the cavity and later give rise to paraphyses and sex organs. Meanwhile unaffected epidermal cells continue division and cut off basal segments which become part of the cortex. "This new cortical growth stops abruptly at the conceptacle. In this way the cavity is deepened and a neck is formed, this neck being composed of epidermis-like cells. Original cortical rows are slightly deflected around the forming cavity, but later become deeply invaginated and thus aid in deepening the conceptacle" (pp. 35–36).

SIMONS in 1906, in a study of *Sargassum* (**27**), claims a development of conceptacle "At variance with all the prominent characteristics . . . . of the conceptacle as described by BOWER." The initial does not break down, but is an active cell producing the entire conceptacle. Adjacent cortical tissue is in no way involved in the process (p. 169). "Both the conceptacles and cryptostomata originate in a single flask-shaped initial which develops the entire structure. The first division of the initial results in two unlike segments: a large lower cell which develops the walls of the conceptacle and cryptostoma; and an upper cell, the tongue cell, which either remains inactive, divides to form a short filament, or degenerates. The "initial" cell of BOWER is apparently the tongue cell, a product of the true initial cell. The conceptacle and cryptostoma are undoubtedly homologous structures. Every stage of development in both structures is the same, from the appearance of the similar initial cells to the development of paraphyses in the cryptostomata and sexual organs in the conceptacle" (p. 179).

Summary of literature

The various theories described in the foregoing seem to resolve themselves into three categories: (1) the conceptacle or hairpit is a slight modification of the external layer of the thallus; this theory was held by KÜTZING, SACHS, and LUERSSEN (**15**); (2) the conceptacle is a product of one or more initials, which do or do not disintegrate; their basal segments form the basal portion, whereas adjacent cortical tissue completes the sides of the structure; this theory was held by REINKE, BOWER, VALIANTE, OLTMANNS, BARTON, MITCHELL, WHITTING, MURRAY, HOLTZ, FALKENBURG (**7**), and others; (3) the conceptacle is a product of a single initial whose segments develop the entire body; this is the theory of SIMONS. All of these workers agree, more or less, that conceptacles and hairpits are homologous structures.

Description

In a study of three species of *Fucus* and of *Splachnidium rugosum* and a rather superficial examination of *Sargassum filipendula* and *Hormosira*, the writer found evidence which supports the early

claim that the conceptacle is merely a slight modification of the original external layer of the thallus.

Following segmentation of the apical cell, in *Fucus*, some segment on the inner lip of the apical groove, close to the apical cell itself, ceases activity, and through failure to continue growth for a time is left in a depression by the active growth of abutting tissue. This inactive segment may then begin to break down in its external portion, without cutting off a transverse segment beneath to give a "basal cell" (figs. 1–4, 5*c*). Again, a basal segment may be cut off (figs. 5*b*, 6, 11–15), or even two segments may behave in a similar fashion (figs. 8, 9, 10). As the surrounding tissue comes to surpass the original inactive segment, the cells immediately abutting begin to break down in their external portions, as did the original segment (figs. 1–15). By continued involving of outer segments the cavity is gradually enlarged, and since the adjacent segments are always more active and therefore have greater turgor, the neck of the structure is narrow, whereas the basal part is broad.

Meanwhile, more and more of the outer portions of the cells involved break down, becoming mucilaginous and often showing several layers when stained with Lichtgrün (figs. 4, 6, 8, 10, 12, 14, 15). Ultimately, all except the portion immediately surrounding the nucleus having disintegrated, the original segments are left as mere lumps of tissue lining the cavity (figs. 6–15). *These basal portions never lose their meristematic activity*, but after a period of quiescence begin to put forth papillae as the cavity enlarges (figs. 16, 17). These papillae develop into hairs by basipetal segmentation, and finally become mature, multicellular, unbranched hairs which fill the cavity and project beyond the opening, out over the surface of the thallus (figs. 15–18).

Some time after maturity of the hairpit, these hairs are shed, and from the basal portions (*of the original segments*) new papillae put forth, this time to develop into hairs of a more delicate and fragile nature, often lost very shortly after (figs. 18, 19). Up to this stage there is no appreciable difference between hairpit and conceptacle. Moreover, further development merely offers a means of distinguishing antheridial from oogonial conceptacles,

if the species happens not to be monoecious. Immediately upon loss of the fragile hairs described in the foregoing, or from basal portions which failed to develop them, new papillae develop which by their subsequent behavior indicate whether antheridia or oogonia are to come. In the former case, segmentation may continue until an elaborately branched and complicated structure bearing numerous antheridia is formed. In case of formation of oogonia, the papillae segment once, cutting off the pedicels and oogonia proper (fig. 20). Further development up to blocking off of 8 eggs (fig. 20) is as already described by many writers (FARMER and WILLIAMS **9**, OLTMANNS **19** and **20**, STRASBURGER **28**, YAMANOUCHI **30**, SIMONS **27**, and others).

In *Splachnidium rugosum* the cavity is initiated by disintegration of an entire row of the thallus (and a terminal hair as well in some cases), pressure of active cells on each side reducing the disintegrating row to a long filiform structure which is left free subsequently by withdrawal of abutting cortical tissue in its effort to keep pace with active tissue toward the exterior. The abutting tissue at the external surface continues division and active growth, leaving the original initial row in a depression. Reproductive organs succeed a loss of hairs in the pit, just as in *Fucus*, except that here there are no evident antheridia or oogonia, but merely reproductive sacs.

Examination of *Hormosira* and *Sargassum* material seems to show the same behavior.

Discussion

It will be noted from the foregoing description that the *sex organs appear after maturity of the hairpit*, and, *moreover, they appear in the hairpit itself*. In other words, the hairpits and the conceptacles are certainly homologous structures, as already admitted by many, since one, *the hairpit, is the juvenile stage of the other, the conceptacle*. *All hairpits*, therefore, *are potentially capable of producing reproductive organs*.

Moreover, sex organs have actually been seen in hairpits, described by SIMONS (**27**) as a "peculiar condition" (p. 173). She finds papillae and stalked cells like those which precede male

organs in a conceptacle or like the male organs themselves. She considers this condition a proof that hairpits are derived from the conceptacle (p. 174).

The writer is strongly inclined to the belief that those forms which are reported to possess hairpits only, with no evident connection to reproductive organs, will, upon further investigation, show closest relationship to reproductive activity, as already proved in research upon forms of Ectocarpaceae, Laminariaceae, Dictyotaceae, and Cutleriaceae. To the writer the resemblance of *Hydroclathrus* (**16**) to Fucaceae, where it is reported that after shedding sporangia basal cells originate new growth, is startling. In all the cases where similar sori are described, the origin is constantly from modification of the external layer, even when the writers themselves do not emphasize this fact. BOWER'S description of *Halidrys* and *Himanthalia* (**6**) seems to the writer good evidence against his own theory, and the writer is inclined to the opinion that with technique as it is developed today, BOWER would find the conceptacle of *Fucus* of quite the same origin as in *Halidrys* and *Himanthalia*. Even as it stands, several of BOWER'S drawings (pl. 5, figs. 2, 5, 11) show a possibility of interpretation quite like that of the writer.

Sections made by the writer of the same species of *Sargassum* cast some doubt on the explanation offered by SIMONS (**27**). Moreover, sections of young tips of *Fucus vesiculosus* give almost identical stages like those shown in her drawings of *Sargassum* (pl. 10, figs. 1, 3, 5, 6, 12, 17, etc.). However, sections of later stages show subsequent behavior to be quite different from that described by SIMONS, and to be as described by the writer for *Fucus*. It remains for further investigation to prove whether SIMONS is entirely accurate in her description. It seems to the writer that what SIMONS interprets as segments of the basal portion of the "initial" are really the basal portions of original segments of the apical cell, similar in nature and behavior to the "initial."

As to the various terms suggested, BOWER'S "neutral conceptacle" ("sterile conceptacle") and BARTON'S "vegetative conceptacle" are equally unhappy; if the body is a true conceptacle it cannot be "neutral," "sterile," or "vegetative." Since the

modern trend is for simplicity rather than complexity, and since the hairpit is simply an early stage of the conceptacle, the writer is strongly inclined to reject both BOWER'S and BARTON'S terms and retain the simpler term "hairpit," an exact translation of the early German use of "Fasergrübchen." BOWER'S "central column" is explained by examination of figs. 13–15. The old walls become mucilaginous and sometimes several layers of mucilage are seen between the meristematic portion and the "column."

Conclusion

There seems a clear line of advance from forms with continuous patches of hairs and reproductive organs, as *Nereocystis* and allied forms, to distinct sori, where the hairpit is the center of a reproductive group, as in some Ectocarpaceae, Laminariaceae, Cutleriaceae, and Dictyotaceae, to distinct conceptacles scattered over the entire vegetative body, as *Splachnidium*, *Hormosira*, etc., to a final distinct grouping at the apex of a shoot, as in *Fucus*, *Pelvetia*, and other Fucaceae, or on special side branches, as in *Sargassum*, *Turbinaria*, etc. Similar lines of advance are already commonly accepted for bryophytes and pteridophytes, in the one restricted to arrangement of sex organs, in the other to arrangement of sporangia. A striking parallel to *Fucus* is seen in *Corsinia*, *Riccia*, and allied forms, where, step for step, the development of the dorsal groove and subsequent appearance of sex organs (and even hairs) from the basal portion repeats the history of the development of the conceptacle of *Fucus* from an original segmentation of the apical cell. In this one sees a forceful illustration that plants again and again duplicate behavior in given conditions, even though in one case the plant may be a gametophyte and in the other a sporophyte.

Summary

1. The conceptacle originates as a slightly modified continuation of the external layer of the thallus, being segments of the apical cell whose basal portions are constantly meristematic and *never entirely breaking down*.

2. The hairpit is a juvenile stage of the conceptacle, the sex organs appearing in the same cavity as the mature hairs, but after their loss.

3. A distinct phylogenetic series is seen in advance from continuous patches of hairs and reproductive bodies, to scattered sori, to scattered conceptacles, and finally to apically placed conceptacles or to conceptacles on specially developed side branches. All of these structures originate through modification of the superficial layer of the thallus.

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LITERATURE CITED

1. AGARDH, C., Species Algarum. **1**. Gryph. 1823–1828.
2. AGARDH, J. G., Species, genera, et ordines Algarum. **1**. London. 1848.
3. BARTON, E. S., A systematic and structural account of the genus *Turbinaria*. Trans. Linn. Soc. London II Bot. **3**:215–226. *pls. 14, 15*. 1891.
4. ———, On the structure and development of *Soranthera*. Jour. Linn. Soc. Bot. **33**:479–486. *pls. 23, 24*. 1898.
5. ———, On the fruit of *Chnoospora fastigiata*. Jour. Linn. Soc. Bot. **33**: 507–508. *pl. 28*. 1898.
6. BOWER, F. O., On the development of the conceptacle in the Fucaceae. Quart. Jour. Micr. Sc. **20**:36–49. *pl. 5*. 1880.
7. FALKENBURG, P., Die Algen im weitesten Sinne. Schenk's Handbuch der Botanik **2**:159–314. Breslau. 1912.
8. FARLOW, W. G., List of marine algae of the U.S. Proc. Amer. Acad. **10**:351ff. 1875.
9. FARMER, J. B., and WILLIAMS, J. L., Contributions to our knowledge of the Fucaceae; their life history and cytology. Phil. Trans. Roy. Soc. London B **190**:623–645. *pls. 19–24*. 1898.
10. GREVILLE, R. K., Algae britannicae. Edinburgh. 1830.
11. HOLTZ, F. L., Observations on *Pelvetia*. Minn. Bot. Studies **3**:23–45. *pls. 7–12*. 1903.
12. KJELLMAN, in ENGLER und PRANTL, Die natürlichen Pflanzenfamilien 1^2: 1897.
13. KÜTZING, F. T., Phycologia generalis. Leipzig. 1843.
14. LEITGEB, H., Untersuchungen über die Lebermoose, Heft IV. Die Ricieen. *pls. 1–9*. 1879.
15. LUERSSEN, C., Handbuch der Syst. Bot. **1**:1879.
16. MITCHELL, M. O., On the structure of *Hydroclathrus*. Phyc. Mem. **2**: 53–56. *pl. 14*. 1893.

17. MITCHELL, M. O., and WHITTING, F. G., On *Splachnidium rugosum* Grev., the type of a new order of algae. Phyc. Mem. **1**:1–10. *pls. 1–3.* 1892.
18. MURRAY, G., On the cryptostomata of *Adenocystis*, *Alaria*, and *Sacchorhiza*. Phyc. Mem. **2**:59–63. *pl. 16.* 1893.
19. OLTMANNS, F., Beiträge zur Kenntniss der Fucaceen. Cassel. 1889.
20. ———, Morphologie und Biologie der Algen. Jena. 1905.
21. REINKE, J., Über *Fucus vesiculosus*. Bot. Zeit. **33**:463–470. 1875; Nachricht K. Gesells. Wiss. Göttingen. 230–241. 1875; Jahrb. Wiss. Bot. **10**:317. 1876.
22. ———, Beiträge zur Kenntniss der Tange. Jahrb. Wiss. Bot. **10**:317–382. 1876.
23. ———, Entwickelungsgeschichte Untersuchungen über die Dictyotaceen des Golfs von Neapel. Nov. Act. Leopold **50**:1878.
24. ———, Atlas deutscher Meeresalgen. Berlin. 1889–1892.
25. ROSTAFINSKI, J. T., Beiträge zur Kenntniss der Tange. **1**. Leipzig. 1876.
26. SACHS, J., Lehrbuch der Botanik. Eng. ed. Oxford. 1875 (pp. 226–229).
27. SIMONS, E. B., Morphological study of *Sargassum filipendula*. BOT. GAZ. **49**:161–182. *pls. 10, 11.* 1906.
28. STRASBURGER, E., Kerntheilung und Befruchtung bei *Fucus*. Jahrb. Wiss. Bot. **30**:351 seq. 1897.
29. VALIANTE, R., Le Cystoseirae del Golfo di Napoli. Fauna und Flora. Leipzig. *pls. 1–15.* 1883.
30. YAMANOUCHI, S., Mitosis in *Fucus*. BOT. GAZ. **47**:173–197. *pls. 8–11.* 1909.

EXPLANATION OF PLATES XIV–XVII

The drawings were all made with the aid of the Abbé camera and reduced one-half in reproduction. For figs. 1–18, the original magnification was 1050; for figs. 19–20, the original magnification was 535. Figs. 1–19 are of *Fucus edentatus;* fig. 20 is of *Fucus vesiculosus.*

FIGS. 1*a*–20*a*.—Sketches to show topography.

FIG. 1.—The inactive segment of the apical cell; the external portion already shows a breaking down; adjacent cells also show inactivity in a similar way.

FIGS. 2–4.—Further breaking down.

FIG. 5.—(*b*) Original segment which has cut off a basal segment; (*c*) original segment with no such segment cut off.

FIGS. 6, 7.—Further stages of figs. 1–3.

FIGS. 8–10. Two or more arrested segments.

FIG. 11.—Further stage of figs. 1–7; basal portions about nuclei very distinct.

FIG. 12.—Further breaking down; involving of abutting cells especially clear.

FIGS. 13, 14.—Showing how old walls are attached to "central column."

FIG. 15.—Fragments in center due to disintegration of walls still attached to column in figs. 13, 14.

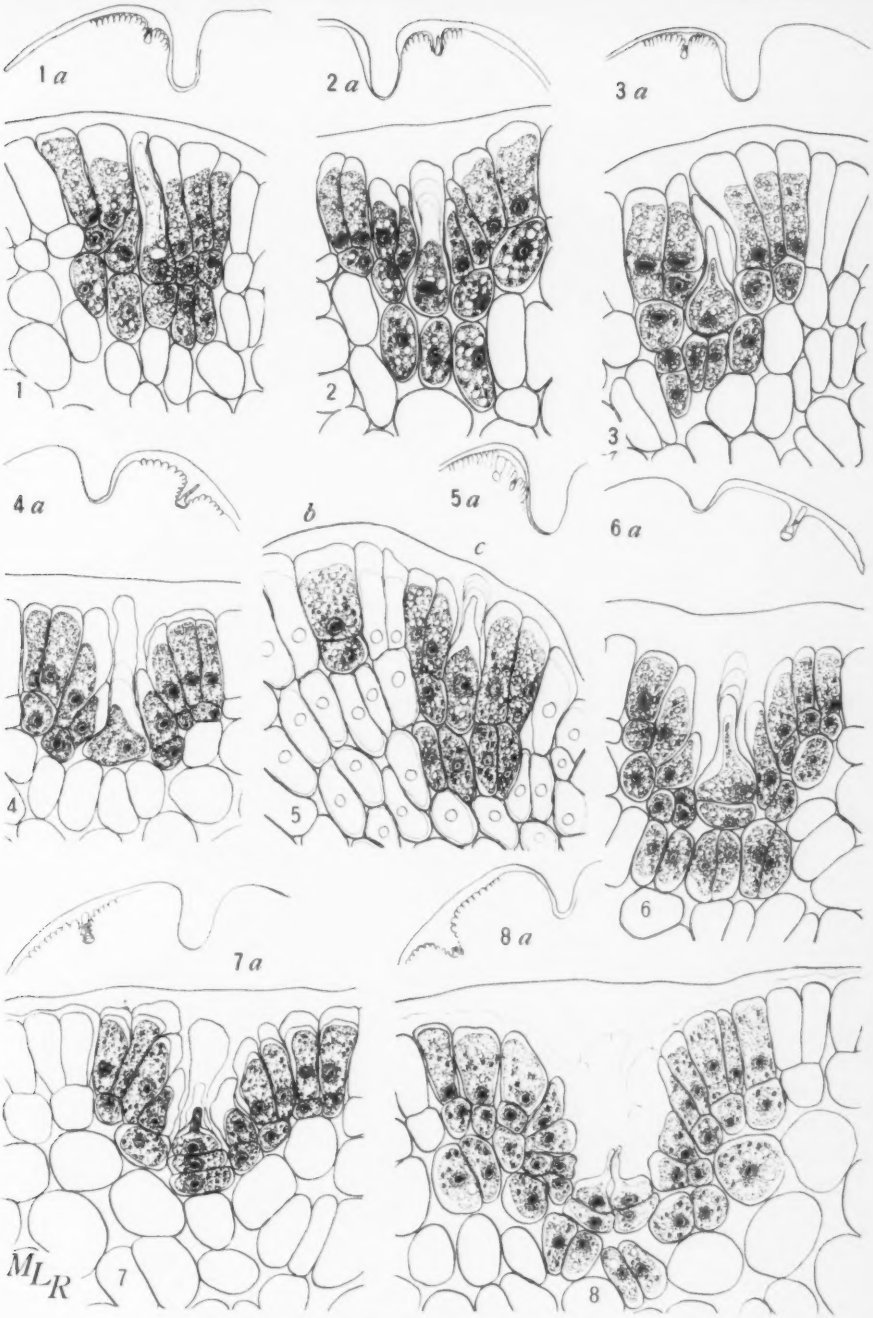
FIG. 16.—Young hairs developed from meristematic basal portions of original segments.

FIG. 17.—Older stage of same.

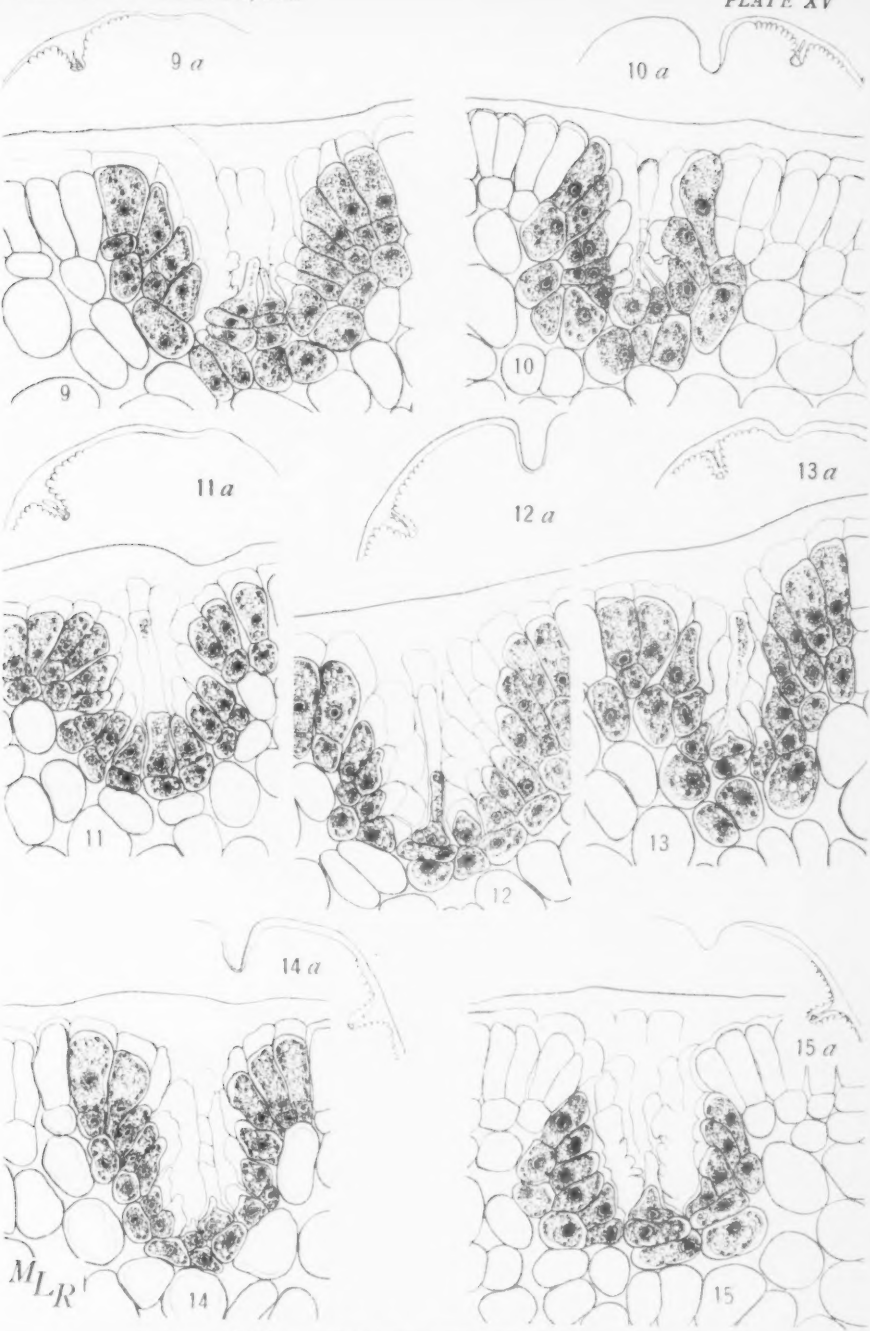
FIG. 18.—Mature hairpit; meristematic portions of segments seen on side with papillae.

FIG. 19.—Older pit; long hairs falling off; meristematic portions of segments putting forth papillae and delicate hairs.

FIG. 20.—Mature conceptacle; delicate hairs being shed; oogonia in all stages from one-celled to complete blocking off of 8 eggs, developed by papillation and subsequent segmentation from meristematic portions of original segments; old hairs still seen about the ostiole, as well as fragments scattered through the cavity.



ROE on CONCEPTACLE OF FUCUS



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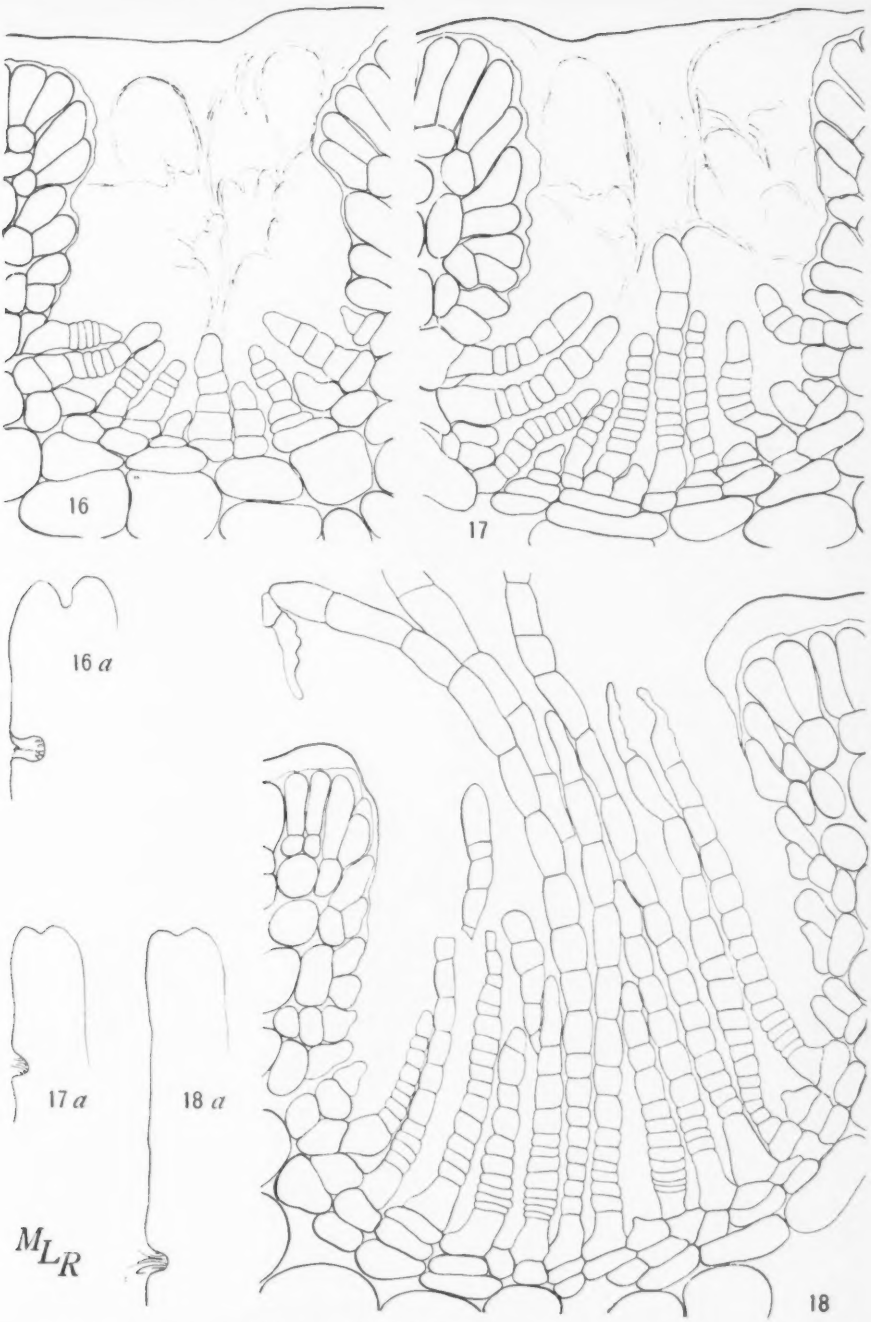

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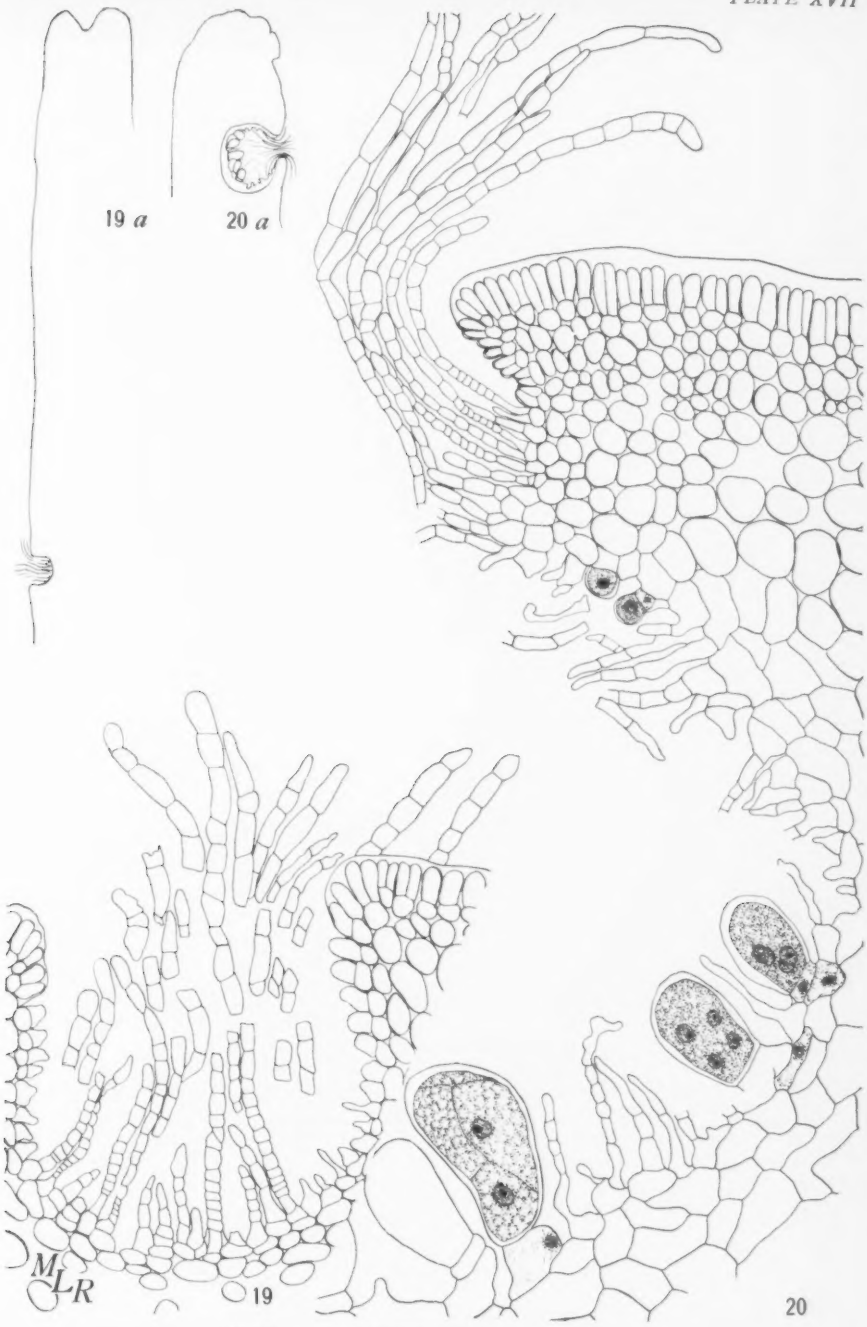

ROE on CONCEPTACLE OF FUCUS

A NEW METHOD OF SEPARATING FUNGI FROM PROTOZOA AND BACTERIA

NICHOLAS KOPELOFF, H. CLAY LINT, AND DAVID A. COLEMAN

The investigations of RUSSELL and HUTCHINSON,[1] GOODEY,[2] CUNNINGHAM and LÖHNIS,[3] and others have stimulated the development of a new branch of soil biology, namely, soil protozoology. A problem of considerable interest in this field is the determination of the effect of soil protozoa upon soil bacteria. RUSSELL and HUTCHINSON maintain that soil protozoa have a marked influence on bacterial activity, and consequently soil protozoa may be regarded as one of the limiting factors in soil fertility. The criterion for measuring the effect of soil protozoa on bacterial activity has commonly been the production of ammonia and nitrates.

In pursuing this problem, some preliminary experiments, one of which was concerned with soil fungi, were carried on by the writers. In view of the fact that fungi are capable of producing ammonia,[4] their presence might mean an additional factor not accounted for in measuring the effect of soil protozoa on soil bacteria. So far as we have been able to ascertain, neither RUSSELL and HUTCHINSON nor other investigators dealing with similar problems have taken into consideration the possible value of this factor. Fungi are capable of producing ammonia, and until it has been established that they do not alter the results of ammonia production in the presence of bacteria in such experimental work, it would seem a priori that their presence is undesirable. Since a survey of the literature bearing on the subject offered neither suggestions nor a solution of the difficulty, the authors attempted to devise a method for the elimination of this factor.

[1] RUSSELL and HUTCHINSON, Jour. Agric. Sci. **3**:111. 1909; **5**:152. 1913.

[2] GOODEY, Proc. Roy. Sci. London **84**:165. 1911.

[3] CUNNINGHAM and LÖHNIS, Centralbl. f. Bakt. II. **39**:506. 1913; **42**:8. 1914.

[4] MÜNTZ and COUDON, Compt. Rend. **116**:395. 1803.
MARCHAL, Bull. Acad. Sci. Belg. III. **25**:727. 1803.
MCLEAN and WILSON, N.J. Bull. 270. 1915.

The method finally devised and tested is based upon the principle of dilution, in such a manner as to reduce the possibilities for the occurrence of fungi in the cultures. Poured agar plates were used for this purpose. The method of procedure was as follows:

SERIES 1.—Plates of 10 different agar media suitable for growing fungi were poured in duplicate. They were potato, oat, cornmeal, rice, bean, raisin, appie, synthetic (LIPMAN and BROWN, N.J. Ann. Rep. 1908. p. 133), soil extract agar (prepared by adding 15 gm. agar to 1000 cc. Löhnis soil extract), and Cook's fungi medium no. II (Del Bull. 91. p. 10).

After cooling, a block of each medium about 2 cm. square was cut out with a sterile knife, and 1 cc. of sterile soil extract was introduced by means of a sterile pipette into the cavity formed. A platinum loopful of a 3-day old culture of soil organisms in soil extract, known to contain numerous bacteria, protozoa, and fungi, was then carefully rinsed off in the medium. This soil extract was prepared according to Löhnis' directions by heating 1 kg. of good soil with 1 liter of water at 15 lbs. pressure in the autoclave half an hour. It was then removed, mixed with a generous quantity of talc, shaken thoroughly, and the liquid filtered through a double thickness of filter paper, until a clear solution was obtained. The moist residue of soil in the flask was pressed out to remove any solution still remaining. The solution was made up to a volume of 800 cc. and 0.05 per cent K_2HPO_4 added.

SERIES 2.—This served as a check on series 1, consisting of poured plates each inoculated with one loopful of the same 3-day old culture of organisms, and made at the same time, using the 10 different media previously mentioned.

SERIES 3.—After one week, poured plate cultures were made, using the same media and inoculating with one loopful of the solution taken from the cavities of the agar plates of series 1, in order to make doubly certain that fungi were not present.

The results in series 1 show that on the plates where a portion of the agar was removed and 1 cc. of soil extract substituted, the bacteria and protozoa developed in enormous numbers, which might be due to the fact that a large surface is exposed for such a relatively small quantity of media. The important point, however,

which is to be noted from this experiment, is that despite the fact that media were furnished for the growth of fungi none was evident, even after 30 days' incubation.

From the observation of the poured plate cultures of series 2, made from the same 3-day old culture as series 1, it was noted that fungi appeared after 4 days upon 3 out of 10 plates; namely, Cook's no. II, Lipman and Brown's synthetic, and raisin agar. The predominating fungi were species of *Penicillium*, *Alternaria*, and *Fusarium*. On the poured plate cultures of series 3, inoculated with the solution in the cavities of the agar plates in series 1, no fungi developed. This experiment was repeated and corroborated the previous results. Thus it appears that although fungi were present in the original culture, the process of high dilution was responsible for their elimination in the specially prepared cavity on the agar plates in series 1.

Another method with the same object in view, namely, the separation of fungi from bacteria and protozoa, was employed, the procedure of which was as follows: Poured plate cultures of the 10 different media (as before) were made from the same 3-day old culture of soil extract known to contain numbers of bacteria, protozoa, and fungi, and the plates were watched carefully throughout the period of one week's incubation for the appearance of any fungi. As soon as their presence was discerned, the fungous growths were removed with a sterile scalpel. At the end of one week, at which time it was reasonably certain that the fungi had ample opportunity to develop, a portion of the agar, about 1 sq. in. in size, was removed with a sterile scalpel from each of the 10 plates, placed in 50 cc. of soil extract, and the flask thoroughly shaken to disintegrate the agar. For 4 days a microscopic observation was made and a few small flagellates and small ciliates were discovered on the preparation from raisin agar, and a few small ciliates and numerous small flagellates on the apple agar. No large ciliates were noted.

Summary

1. The dilution method followed by the peculiar manner of plating as outlined makes it possible to separate fungi from bacteria and protozoa.

2. As a result of this separation it is possible to eliminate fungi from experiments involving the effect of protozoa upon bacterial activity, by making a subculture from the fungi-free solution of bacteria and protozoa (in the cavity of the agar plate).

3. The second method described, that of removing the fungi from the plates as they appeared, is undesirable for our special investigation, for the reason that the bacteria are allowed to multiply easily, while the protozoa have no such favorable conditions; consequently, on transferring such a culture to the soil, the protozoa would be at a considerable disadvantage, and their activity would be seriously inhibited if not entirely suppressed. The suggestion may be offered, however, that this method might be employed for obtaining cultures of single types of protozoa, as for example small flagellates or small ciliates.

This paper represents one phase of the preliminary work undertaken in connection with an investigation of the effect of soil protozoa upon the activity of soil bacteria. Further results on experimentation and a bibliography on soil protozoa and soil sterilization are awaiting publication.

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BRIEFER ARTICLES

CHLOROFORM AS A PARAFFIN SOLVENT IN THE IMBEDDING PROCESS

In a recent number of this journal LAND[1] describes an improved method of replacing the paraffin solvent with paraffin, in which he calls attention to certain difficulties encountered in the use of xylol as a solvent, and offers some suggestions by which these difficulties may be overcome.

The writer is somewhat surprised to find that xylol is so extensively used as a paraffin solvent in the process of infiltration, and for this reason he is tempted to describe the method that he has used for a number of years. In the first place, judging from the writer's experience, the best way, or at least one of the best ways, to avoid the difficulties mentioned by LAND in the use of xylol is to do away with the xylol altogether. Nineteen years ago the writer discarded the use of xylol. In its place he used chloroform, and he has been convinced that this solvent is superior to xylol. His method is as follows: We assume that the material has been carefully and thoroughly dehydrated by passing through the grades of alcohol, beginning with very low percentages, depending upon the character of the tissue. In dehydrating very soft objects one may begin by adding a few drops of alcohol at a time until a strength of 10 or 15 per cent has been reached. I have found this a safe thing to do in the case of objects like the seaweed *Champia parvula*. Some objects, however, may be placed directly from water into 20 per cent alcohol. It does not seem that standing overnight in any of the weaker grades of alcohol injures the tissues in the least.

After the specimens have been thoroughly dehydrated (I refer chiefly to material for morphological and cytological purposes), they are placed in a mixture of equal parts of absolute alcohol and chloroform, where they should remain for 2–3 hours or longer. When thrown into this fluid the specimens float, but in a short time they sink to the bottom of the vessel. No injury results if the specimen be allowed to remain in the chloroform and alcohol overnight or longer. Next the specimens are placed in pure chloroform, where they remain for 2–12 hours, depending upon the size of the specimens, the nature of the tissue, and to some

[1] LAND, W. J. G., Microtechnical methods. BOT. GAZ. **59**:397–401. 1915.

extent upon the composition of the fixing or killing reagent. Generally the material is left in the pure chloroform 2 hours, or until it sinks to the bottom of the vessel. Material such as root tips or lily anthers fixed in chromo-osmic-acetic acid or chromo-osmic acid will sink in pure chloroform within 2 or 2.5 hours, but if chromo-acetic acid, absolute alcohol, or reagents that do not contain osmic acid are used as killing reagents, the specimens sink slowly or not at all. In the case of material fixed in chromo-acetic acid, for example, the specimens are left in the pure chloroform overnight or longer if necessary.

The specimens are now changed to a fresh quantity of pure chloroform, and shavings of paraffin (melting point 42–45°) are added until at room temperature no more paraffin will dissolve. It will be seen that the paraffin floats at the surface of the fluid, while the specimens, in case they have sunk, are at the bottom. The chloroform surrounding the objects becomes gradually saturated, therefore, at room temperature. If the objects have not sunk in the chloroform, they do so gradually as the paraffin is dissolved. The degree of saturation of the solution may be increased slowly by adding paraffin a little at a time, but the writer has not found any special care necessary. When the chloroform is saturated at room temperature, the vessel is placed upon the paraffin oven and a little more paraffin is added if desired. The vessel, which is still closed with a stopper, remains on the oven 2–12 hours. The contents are now poured out into an open dish (usually a small porcelain dish) and covered by only a slip of paper to keep out dust, and this dish remains upon the oven until so much chloroform evaporates that the paraffin congeals slightly at the edge of the dish or over the whole surface. This requires usually one night. The dish is then placed inside the oven and allowed to remain until all the chloroform has evaporated, as determined by taste. The specimens are now transferred to melted paraffin of 52–55° melting point, or that of a higher melting point if necessary, in which they remain 10 minutes to 2 hours or longer before imbedding. They are then imbedded in this or similar paraffin.

Although chloroform is expensive, this is not necessarily a costly process. The paraffin (45° melting point) used in making the chloroform-paraffin solution may be used over again two or three times, and 8 cc. of chloroform in each vessel is sufficient for a quantity equal to 10 or 15 root tips of onion or lily anthers.

It is understood, of course, that chloroform should be kept out of direct sunlight, preferably in a dark place.—D. M. MOTTIER, *Indiana University, Bloomington, Ind.*

In the Hull Botanical Laboratory, after a long series of rigid comparative tests of the various paraffin solvents in general use, it was found that xylol when carefully used gave uniformly better results than any other solvent. Cedar oil was rejected because it is almost if not quite impossible to eliminate the oil in the final stages of imbedding. It is true that hard material cuts somewhat better after cedar oil, but the same end may be attained in a far better way by soaking the imbedded material in water.

Chloroform was abandoned because in transferring from alcohol to chloroform it was found that, even when a much closer series than the one recommended by Professor MOTTIER is used, some plasmolysis results. Also we have found that paraffin does not seem to penetrate the tissues as readily after chloroform as after xylol. These results should be expected when we remember that the specific density of chloroform is nearly twice that of either alcohol, xylol, or paraffin. In STRASBURGER's laboratory chloroform was practically abandoned for xylol about 15 years ago. We have in this laboratory preparations of root tips as well as of *Lilium* anthers showing reduction division, which, from the maker's name, we assume were made exactly as described by Professor MOTTIER. These preparations are certainly inferior to those in which xylol was used as a solvent. Dr. L. W. SHARP, one of the most successful workers in the peculiarly difficult field of modern cytological technique, always uses xylol as a solvent.

If all stages in cytological technique received equal care, published results would undoubtedly be in closer accord than they are at present.—W. J. G. LAND, *University of Chicago.*

BESSEYOSPHAERA, A NEW GENUS OF THE VOLVOCACEAE

Two new species of the Volvocaceae were described by POWERS[2] without names or assignments to taxonomic positions. They were designated "first form of *Volvox*" and "second form of *Volvox*." The former was subsequently further described and named *Volvox spermatosphaera*.[3] The "second form" is intermediate between *Pleodorina* and *Volvox* in the scale of differentiation, and its assignment to either genus would involve so great an extension of the conception of the

[2] POWERS, J. H., New forms of *Volvox*. Trans. Amer. Mic. Soc. **27**:123–149. 1907.

[3] ———, Further studies in *Volvox*. Trans. Amer. Mic. Soc. **28**:141–175. 1908.

genus as to be undesirable. Its nearest affinity may be considered to be *Pleodorina californica*, originally described by the writer[4] and more completely described by CHATTON.[5] I now propose to treat this "second form" as the type of a new genus, dedicated under the name *Besseyosphaera*, to the memory of the late Professor CHARLES E. BESSEY.

This new genus is distinguished from *Pleodorina* chiefly by having the gonidia scattered among the vegetative cells, instead of being developed from all the cells of the reproductive area. It differs from typical *Volvox* in having no protoplasmic connections between the cells, and in the lateness of the first visible differentiation of the gonidia, which does not appear until after birth. A study, now in progress, of several species of *Volvox* collected in the Philippine Islands leads us to anticipate that species lacking the intercellular protoplasmic connections may be properly removed from *Volvox*, some ranking below and some above *Volvox* in the scale of development. One or more of these may prove to belong properly in the same genus with the subject of these notes. For the present, however, it is convenient to treat our new genus as monotypic. Taking as a basis the facts stated by POWERS,[6] and making assumptions which seem to be warranted by the use of the name *Volvox* for this organism, the following diagnosis is offered.

Besseyosphaera, gen. nov.—Body a hollow spherical "coenobium" of greenish biciliate cells which lie in the periphery of a gelatinous matrix surrounded by a common hyaline envelope. Gonidia developed from cells distributed among the vegetative cells. No intercellular protoplasmic filaments. Daughters born before differentiation of gonidia. Sexual reproduction not known.

B. Powersi, sp. nov.—*Second form of Volvox*, J. H. POWERS, Trans. Amer. Mic. Soc. **27**: 140–144. *pl. 14. figs. 19–24.* 1907.—Number of cells in the body about 1000 ("often below," "seldom much above"). Maximum diameter of body about 2000 μ (1800–2500 μ recorded). Vegetative cells about 12 μ in diameter; separated by 50–200 μ. Gonidia 10 or more to 78 or more; distributed in two-thirds or four-fifths of the surface of the body; differentiated in the daughters after birth. Daughters less than 150 μ in diameter at birth. Sexual reproduction not known. Habitat not stated (presumably a fresh water pond in Nebraska, United States).—WALTER R. SHAW, *University of the Philippines, Manila, P.I.*

[4] SHAW, W. R., *Pleodorina*, a new genus of the Volvocineae. BOT. GAZ. **19**: 279–283. 1894.

[5] CHATTON, E., *Pleodorina californica* à Banyul-surmer: son cycle évolutive et sa signification phylogénetique. Bull. Sci. France et Belg. **44**: 309–331. 1911.

[6] *Loc. cit.* 1907.

A METHOD FOR THE DEHYDRATION OF HISTOLOGICAL MATERIAL

A combination of the glycerine dehydration method with the paraffin imbedding method in preparing histological material has given such satisfactory results that, while there is nothing new in the method itself, its advantages seem to justify bringing it to the attention of botanists, since the combination of the two is not in general practice. For some time the writer has been using glycerine in dehydration instead of alcohol in the preparation of small objects such as the megaspores and microspores of *Marsilia* and *Selaginella*, and has recently attempted using it on larger objects such as leaf tissue, ovulate and staminate sporophylls of the conifers, and anatomical material generally. It has uniformly given good results and indicates possibilities of more general application.

The method is exactly the same as is given for the glycerine dehydration in the preparation of glycerine and Venetian turpentine mounts of algae and fungi. The material to be imbedded is first killed with some of the usual killing agents, such as Fleming's solution or chromo-acetic acid solution 1:1:100 diluted to one-third or one-fourth strength at a temperature of 50–60°C. After killing for 12–24 hours the usual washing with water should follow. When free from acid the material is placed in a shallow open container, as a watch glass or a Petri dish, and covered with a 10 per cent glycerine solution in sufficient quantity to more than cover the material. The dish is allowed to stand open, but protected from the dust and subjected to the ordinary evaporation of the laboratory air, insuring a steady rapid dehydration by the evaporating of the water from the glycerine solution which gradually becomes concentrated. Within two or three days the glycerine is fully concentrated and the material is at about the same stage of concentration it would have been had it been "run up" through grades of alcohol to about 95 per cent.

The glycerine should be rather carefully removed by washing the material in 95 per cent alcohol. Specific manipulation for this particular process may be devised for each kind of material. Large pieces of material may often be removed from the glycerine with forceps or needles, or frequently the glycerine may be poured off the material. In any event, it is important to remove all of the glycerine by repeated washings in 95 per cent alcohol, since the presence of the former seems to interfere in the further processes of imbedding. Absolute alcohol is used to complete the dehydration, and any of the standard methods of substituting a paraffin solvent for the alcohol may follow from this point.

The advantages of the method are as follows: (1) dehydration is accomplished uniformly, rapidly, and with a minimum of work and

attention on the part of the operator; (2) when imbedded by this method material seems to cut better, since glycerine seems to harden less than alcohol; (3) material may be stored in the concentrated glycerine if it be desirable to postpone the imbedding processes.—J. BEN HILL, *Pennsylvania State College, State College, Pa.*

BISPORANGIATE CONES OF LARIX

(WITH ONE FIGURE)

In the early spring of the present year (1915), in the vicinity of Missoula, Montana, abnormal cones were observed among normal ones on a single tree of *Larix occidentalis*. The abnormal cones were about the same size as the normal ovulate cones of the species, and consisted of two parts; the lower half of the cone presented the normal appearance of the staminate cone with the total absence of the scale, the upper half presented the typical condition of the macrostrobilus with bract and scale. The scales bore two ovules, to all external appearances normal. Between the lower and the upper portions of the cone were a few transitional structures representing abortive bracts and scales. Some of these structures are shown in the accompanying figure.

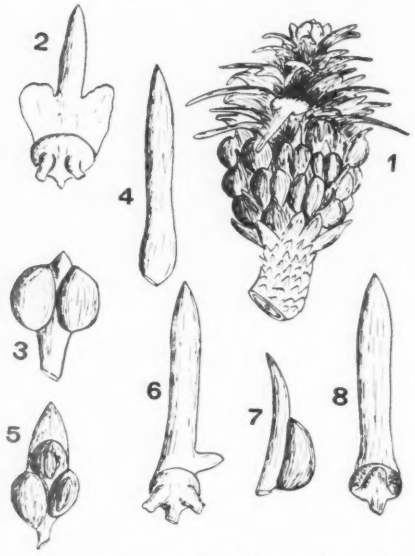

FIG. 1.—*Larix occidentalis:* 1, abnormal or bisporangiate cone, $\times 3\frac{1}{3}$; 2 and 3, normal sporophylls and bract from the cone at 1; 4–8, abnormal bracts; 2–8$\times 6\frac{2}{3}$.

The pollen produced in the bisporangiate cone, on those sporophylls where the sporangia were apparently perfect, in its microscopic structure appeared to be the same as the pollen formed in the normal staminate cone on the same branch, except that it was somewhat smaller, measur-

ing on an average 93 μ in the diameter of the grain, as contrasted with an average of 100 μ in the latter case. In the abnormal sporophylls the pollen was sometimes in lumpy masses and imperfect.

Similar cones have been described in the case of *Picea*, *Sequoia*, and *Pinus*,[7] and have been interpreted as indicating a homology between the bract of the ovuliferous cone and the stamen, a conclusion which seems supported here.—J. E. KIRKWOOD, *University of Montana, Missoula, Mont.*

[7] COULTER, JOHN M., and CHAMBERLAIN, CHARLES J., Morphology of gymnosperms. Chicago. 1910.

CURRENT LITERATURE

BOOK REVIEWS

Books on coal

In view of the increasing importance of the botanical sciences in connection with the fundamental problems, a general statement in regard to the status of plants in relation to coal will not be without interest to the readers of this journal. Taking first the conservational aspect of the subject, the results of the geological congress held in 1913 in Toronto, Canada, have appeared in three magnificent quarto volumes and a large folio atlas.[1]

Although the coal resources of the world are still abundant and exhaustion is in general hundreds of years in the future, still, prudent exploitation of the coal fields is necessary. The account of the coal resources of the various countries of the world are given authoritatively by their geological surveys and bureaus of mines. It is particularly fortunate that this should have happened before the great war has exacerbated international relations to such an extent that cooperation of the kind manifested in this gigantic work is for a long time to come impossible. The summary of the results is made by members of the Geological Survey of Canada. Of particular interest to North Americans is the enormous richness of our coal resources, which, if we include both the United States and Canada, are nearly twice as great as all of the rest of the world combined. In view of the absolutely fundamental relation of coal to modern industrial development the significance of the statistical situation for the northern America continental area can scarcely be overestimated.

The most important recent work on the historical aspects of coal investigation is unquestionably that of STEVENSON.[2] The author has had prolonged personal experience in connection with the coal deposits of the eastern United States, and has visited a number of the more interesting European formations. The summary of the literature on the subject is admirable, and enables one to realize what an enormously large amount of scientific effort has been expended on the investigation of coal during the past hundred years or more. Two main hypotheses of coal formation have held the ground during that period, namely the autocthonous or *in situ* hypothesis, which attributes to coal the same conditions of formation as ordinary peat; and the allocthonous or transport hypothesis, which regards coal as a sedimentary rock formed in

[1] The coal resources of the world. Toronto: Morang & Co. Ltd. 1913. $25.00.

[2] STEVENSON, J. J., Formation of coal beds. Lancaster (Penn.): New Era Printing Co. 1911–1913. $3.50.

open water. The first hypothesis is almost universally accepted by geologists and has received its main support from German investigations. The transport theory of the origin of coal has always been strongly held in France. The author is clearly in favor of the peat hypothesis (*in situ* or autocthonous theory) and supports his views not only by a summary of the literature, commendable on account of his generous fairness, but also as a result of his own observations carried on during many years in the eastern United States.

We may next consider a work on the recent formations of vegetable deposits comparable to coal at once monumental in extent and in view of the recent decease of its author actually a monument.[3] POTONIÉ divides recent deposits into the so-called Sapropelites, the Humus-Bildungen, and the Liptobioliths. The first are deposits formed under open water by wind and water transport, and correspond to the lacustrine deposits of the allocthonous hypothesis of the origin of coal. The author is far from denying the actual existence of large amounts of vegetable material laid down in open water, and frankly admits that in the tropics, where the ravages of fungi in the case of plant matter not permanently submerged are extremely rapid, such accumulation is the only method of importance. In the case of temperate regions, however, as represented by northern Europe, the true peat (Humus-Bildungen) predominates, constituting extensive bogs, moors, and tundras. POTONIÉ has made a considerable study of the plant population of peat moors, and his results will doubtless be of great interest to students of plant geography and ecology. Liptobioliths are the persistent, resinous, waxy, or cutinous remains of plants which survive under the most unfavorable conditions as a result of their resistance to the organisms of decay. The author comes to the conclusion, as a result of his studies of recent accumulations of vegetable matter, that coal has been formed for the most part from autocthonous peat (Humus-Bildungen), and thus puts himself in line with the hypothesis most generally acceptable to geologists at the present time.

An American work dealing with the subject of coal of quite unusual interest and significance is a recent bulletin of the United States Bureau of Mines.[4] This work represents the cooperation, as rare as it is desirable, of a geologist, a botanist specially qualified in connection with the study of peat, and a histologist trained in the laboratories of the University of Chicago, who has given special attention to the actual organization of coal. The plates accompanying the article are numerous and represent a degree of progress in the difficult technique of the study of coal hitherto not found in any governmental publication. On account of the different points of view of the authors the results are not altogether harmonious. Perhaps the most interesting data,

[3] POTONIÉ, H., Die rezenten Kaustobiolithe und ihre Lagerstaetten. 3 vols. Berlin. 1908, 1911, and 1912. M. 8, 10, and 14 respectively.

[4] WHITE, DAVID, DAVIS, C. A., and THIESSEN, R., The origin of coal. Bull. 38, U.S. Bureau of Mines. 1915.

next to those put forward by THIESSEN in connection with the microscopic study of coal, are those of WHITE in regard to the formation of anthracites as the result rather of thrust action than of heat devolatilization, and of DAVIS as to the origin of vegetable accumulations in the United States, which he considers to have been formed mainly under open water. DAVIS' conclusions in regard to the origin of our peat accumulations are all the more interesting as he accepts the orthodox geological view of German origin in regard to the formation of coals from humic matter or peat. It is apparently not without significance that in a country of the extent of the United States, which today is neither extremely cold toward the north nor extends into the tropical regions in the south, the most important accumulations of vegetable matter in nature are not in peat bogs, but in the depths of open waters. A stronger argument derived from the conditions of the present for the aquatic origin of combustible minerals could scarcely be advanced.

It seems clear that improvements in botanical technique have brought within sight the settlement of the long dispute in regard to the mode of origin of what must be regarded both as the most valuable and the most abundant of all minerals. Coal is the universal industrial energy-producing and deoxidizing agent, since it is the only considerable mineral substance of natural occurrence which is not combined with large quantities of oxygen. It will be of interest to follow the investigations, now rendered possible, which will tend to establish a relation between the organization of coal and its industrial utilization in connection with the development of power, the manufacture of oil, gas, coke, dyes, antiseptics, high explosives, lampblack, electric carbons, etc.—E. C. JEFFREY.

NOTES FOR STUDENTS

Current taxonomic literature.—J. A. NIEUWLAND (Am. Mid. Nat. **3**:265–270. 1914) has described 4 new species of *Lythrum* from the Central and Southern states.—V. NORLIND (Rep. Sp. Nov. **13**:401–403. 1914) has published two new species of *Polygala* from Brazil.—F. OSTERMEYER (*ibid.* 395) records a new *Cochlospermum* (*C. Zahlbruckneri*) from Argentina.—N. PATOUILLARD (Bull. Soc. Mycol. France **30**:345–354. 1914) under the title "Contribution à la Flore Mycologique hypogée du Jura" proposes a new genus (*Stephanospora*) based on *Hydnangium carotaecolor* Berk. & Br.—J. PERKINS (Eng. & Prantl. Nat. Pflanzenf. Ergänzungsheft III, zu II–IV für die Jahre 1905–1912, p. 94. 1914) has proposed the name *Carnegicodoxa* for *Carnegia* Perk., not Britt. & Rose.—F. PETRAK (Ann. Mycologici **12**:471–479. 1914) under the title "Beiträge zur Pilzflora von Mähren und Österr.-Schlesien" includes the description of two new genera, namely *Herpotrichiella* and *Leptomassaria*.—R. PILGER (Notizblatt Königl. Bot. Gart. u. Mus. Berlin **6**:109–212. 1914) in cooperation with several specialists under the title "Plantae Uleanae novae vel minus cognitae" has published about 130 new species of Pteridophyta and Spermatophyta from South America based primarily on the collections of

E. Ule from the region of the Amazon. The following new genera are proposed: *Sohnreyia* Krause of the Rutaceae, *Spirotheca* Ulbrich of the Bombaceae, and *Lychniothyrsus* Lindau of the Acanthaceae.—H. Pittier (Rep. Sp. Nov. **13**:312–320. 1914) has published 12 new species of Malvales from Central America. Two new genera are included, namely *Goethalsia* of the Tiliaceae and *Gyranthera* of the Bombacaceae.—J. A. Purpus (Monats. für Kakteenkunde **24**:65, 66. 1914) describes and illustrates a new species of *Echeveria* (*E. leucotricha*) from Mexico.—L. Quehl (*ibid.* 114–118, 158) describes and illustrates two new species of *Mamillaria* from Mexico.—L. Radlkoffer and J. F. Rock (Terr. Hawaii, Board of Agr. and Forestry, Div. Forest. Bot. Bull. no. 1, pp. 1–14. 1911) under the title "New and noteworthy Hawaiian Plants" have published several new species of orchids and include a new genus (*Hibiscadelphus*) of the Malvaceae.—R. A. Rolfe (Kew Bull. 1914. p. 210) has published 3 new species of flowering plants from Costa Rica and Peru. The same author (Curtis' Bot. Mag. *pl. 8551.* 1914) describes and illustrates a new orchid (*Epidendrum profusum*) from Mexico.—C. O. Rosendahl (Bot. Jahrb. **50**:375–397. 1914. Supplement-Band) presents a revision of the genus *Mitella*, recognizing 12 species, one of which is new to science.—W. E. Safford (Jour. Wash. Acad. Sci. **4**:356–368. 1914) presents a paper on "*Acacia cornigera* and its allies" and describes 10 new species.—J. H. Schaffner (Ohio State Univ. Bull. **18**:127–247. 1914) has issued a "Catalog of Ohio vascular plants" in which he lists 2065 species for the state of Ohio.—J. Schiller (Sitzungsber. K. Akad. Wiss. **122**:621–630. 1913) under the title "Vorläufige Ergebnisse der Phytoplankton Untersuchungen auf den Fahrten S.M.S. Najade in der Adria" describes several species new to science and proposes a new genus (*Cymbomonas*) of the Chlorophyceae.—R. Schlechter (Rep. Sp. Nov. **13**:279–287. 1914) gives a discussion of *Philibertia* and *Funastrum* and makes several new combinations in these genera pertaining mostly to Mexican and South American species. The same author (*ibid.* 438–443) has published 11 new species of Asclepiadaceae from Bolivia, based on collections of Th. Herzog, and (Orchis **8**:18–19, 131–137. *pls. 3, 4.* 1914) describes and illustrates new species of orchids of which 4 are from America.—B. Schröder (Hedwigia **55**:183–223. 1914) in a concluding article on the "Zellpflanzen Ostafrikas" proposes the following new genera of the Scenedesmaceae: *Schmidleia*, *Schroederiella*, and *Victoriella*.—O. E. Schulz (Bot. Jahrb. **50**:176–187. 1914. Supplement-Band) under the title "*Bidens chinensis* (L.) Willd. und verwandte Arten" includes a new species of *Bidens* from Costa Rica.—W. A. Setchell (Univ. Calif. Publ. Bot. **6**:70–152. *pls. 10–16.* 1914) under the title "The *Scinaia* assemblage" presents a synoptical revision of this group. The author recognizes 11 species of *Scinaia* of which 5 are new and 7 species of *Gloiophloea* of which 4 are new to science. One new genus is proposed, namely *Pseudoscinaia*, to which two species are referred, one from California, the other from New Holland.—M. Slosson (Bull. Torr. Bot. Club **41**:307–309. *pl. 7.* 1914) records a new fern (*Adiantum*

rimicola) from Utah.—J. J. SMITH (Bull. Jard. Bot. Buit. II. no. 14, pp. 1–56. 1914) under the heading "Die Orchideen von Java" describes several new species and characterizes a new genus (*Abdominea*).—O. STAPF (Bull. Kew 1914, p. 326) records a new species of *Crataegus* (*C. Lindenii*) from Chiapas, Mexico.—T. STUCKERT (Ann. Conserv. et Jard. Bot. Genève **17**:278–309. 1914) under the general heading of "Beiträge zur Kenntnis der Flora Argentiniens" in cooperation with the eminent agrostologist Prof. E. HACKEL has published a fourth article on the grasses of Argentina in which several species new to science are recorded.—H. and P. SYDOW (Philipp. Jour. Sci. Bot. **8**:265–285. 1913) under "Enumeration of Philippine Fungi" describe 37 new species and raise *Tephrosticta*, hitherto regarded as a subgenus of *Teichosporella*, to generic rank. The same authors (Ann. Mycologici **12**:158–165, 195–204, 545–576. 1914) have published a number of new species of fungi and characterize the following new genera: *Nematostoma* of the Sphaeriaceae found on leaves of *Artemisia vulgaris* L. in northern Japan, and *Theissenula*, *Rizalia*, *Meliolina*, *Pycnoderma*, *Angatia*, *Odontoschizon*, *Manilaea*, *Exotrichum*, and *Psalidosperma* from the Philippine Islands.—F. THEISSEN (Ann. Mycologici **12**:63–75. *pls.* 6, 7. 1914) in an article entitled "Über *Polystomella*, *Microcyclus*, u. a." characterizes the following new genera of fungi: *Polyclypeolum*, *Microcyclella*, *Cyclotheca*, *Cryptopus*, and *Ellisiodothis;* also (Broteria Ser. Bot. **12**:13–33. 1914) a new genus (*Phaeoschiffnerula*) is added to the fungus flora of Brazil. The same author with H. SYDOW (*ibid.* 176–194) under the heading "Dothideazeen-Studien" in addition to critical notes on several species describes 8 additional new genera, namely *Trichodothis*, *Phragmodothis*, *Trabutiella*, *Pyrenobotrys*, *Stalagmites*, *Rehmiodothis*, *Phaeodothiopsis*, and *Parmulina*.—A. THELLUNG (Rep. Sp. Nov. **13**:301–303. 1914) characterizes 6 new varieties and forms of *Lepidium bonariense* L. from South America.—C. TORREND (Broteria Ser. Bot. **12**:53–71. 1914) lists the third century of his "Fungi selecti exsiccati"; several new species are described and one new genus, namely *Botryochora* of the Dothideaceae from Mozambique, is proposed.—A. E. TRAAEN (Nyt Mag. f. Naturv. **52**:19–121. *pl.* 4. 1914) under the title "Untersuchungen über Bodenpilze aus Norwegen" includes the descriptions of two new genera, namely *Geomyces* and *Humicola*.—E. ULE (Bot. Jahrb. **50**:Beibl. no. 114, pp. 1–18. 1914) under the title "Beiträge zur Kenntnis der brasilianischen Manihot-Arten" has published 15 new species.—I. URBAN (Rep. Sp. Nov. **13**:444–459. 1914) has described 24 new species of flowering plants from the West Indies.—W. WEINGART (Monats. für Kakteenkunde **24**:81–84, 123–127. 1914) has published a new species of *Phyllocactus* (*P. Ruestii*) from Honduras and a new *Cereus* (*C. acanthosphaera*) from Mexico.—H. F. WERNHAM (Bull. Kew pp. 63–69. 1914) enumerates the Rubiaceae collected by T. A. SPRAGUE in Venezuela and Colombia in 1898–99 and includes the descriptions of 11 new species. The same author (Jour. Bot. **52**:225–227; 313–316. *pl.* 533. 1914) has published 17 new species of Rubiaceae from Central and South America. One new genus (*Neosabicea*) is added from

Columbia.—E. DE WILDEMAN (Rep. Sp. Nov. **13**:369–384. 1914) has published 35 new species and varieties of flowering plants from Central Africa and characterizes two new genera, namely *Brieya* of the Anonaceae and *Giorgiella* of the Passifloraceae. The same author (Bull. Jard. Bot. Brux. **4**:1–241. 1914) in cooperation with specialists has issued "Additions à la flore du Congo." Several species new to science are included and the following new genera are proposed: *Volutellopsis* and *Gilletia* Torrend of the Mucedinaceae.—G. W. WILSON (Mycologia **6**:192–210. *pls. 135, 136.* 1914) in continuation of his studies on the Peronosporales describes several new species and propose as new genus (*Bremiella*) based on *Peronospora megasperma* A. Berlese.—L. WITTMACK (Bot. Jahrb. **50**:539–555. 1914. Supplement-Band) has published 6 new species of *Solanum* from South America.—N. WORONICHIN (Bull. für Ang. Bot. **7**:431–440. *pl. 120.* 1914) describes and illustrates a new fungus (*Plectodiscella piri*) found in the Kaukasus. The genus is said to represent a new family, namely the Plectodiscelleae.—C. H. WRIGHT (Bull. Kew p. 330. 1914) has published a new species of *Hippeastrum* (*H. Elwesii*) from Argentina. The same author (Curtis' Bot. Mag. *pl. 8553.* 1914) describes and illustrates a new species of *Zephyranthes* (*Z. cardinalis*) from specimens growing in the Kew Gardens but presumably of American origin.—J. M. GREENMAN.

Root nodules.—BOTTOMLEY[5] has investigated the root nodules of *Ceanothus americanus*, to which attention was called by BEAL in 1890. He finds that the nodules are modified lateral roots which increase in size each year by the formation of endogenous outgrowths similar in structure to the primary branch. Each nodule when fully grown shows an apical meristematic zone, an infection zone, a bacterial zone, and a basal zone almost free from bacteria. The bacteria when isolated and grown in pure cultures can fix free nitrogen, and evidently belong to the *Bacillus radicicola* group.

Miss SPRATT[6] has studied the well known root nodules or "coralline roots" of the cycads, and finds that all the genera produce them. They are developed primarily by infection with *Bacillus radicicola*, and at the base of each nodule a whorl of lenticels or a continuous zone of parenchyma is produced. The outer cell walls become pushed apart, and are infected by *Azotobacter*, and under certain conditions by *Anabaena* also. The alga is said to stimulate the phellogen to produce other lenticels, from which a zone of tissue is produced that includes the original outer cells in which the alga and bacteria occur. The algal zone is continuous, and consists of a large air space containing *Anabaena* and *Azotobacter*, which is kept intact by papillate cells traversing it from both inner

[5] BOTTOMLEY, W. B., The root nodules of *Ceanothus americanus*. Ann. Botany **29**:605–610. *pl. 28.* 1915.

[6] SPRATT, ETHEL ROSE, The root nodules of the Cycadaceae. Ann. Botany **29**: 619–626. *pl. 29.* 1915.

and outer tissues. No algal zone has been observed in *Macrozamia*, *Zamia*, *Ceratozamia*, and *Bowenia*, but nodules are produced containing *Bacillus radicicola* and *Azotobacter*. The author states that the cycads are the only nodule-bearing plants known in which four organisms are in symbiotic relationship, namely, two nitrogen-fixing bacteria, an alga, and a cycad.—J. M. C.

Sexual reactions of mucors.—BLAKESLEE[7] has been hybridizing mucors and has secured some interesting results. His (+) and (−) strains have become familiar, and he has shown that the majority of mucors are dioecious. In the experiments presented in this paper he has crossed hermaphroditic species with the sexual races of dioecious forms. It seems that some of these hermaphrodites are heterogamic, showing a constant difference in the size of their gametes, the larger one being presumably female and the smaller one male. A sexual reaction was obtained between the (+) strain of a dioecious mucor and the smaller gamete of the hermaphrodite; and conversely, a similar reaction between the (−) strain of the dioecious form and the larger gamete of the hermaphrodite. The conclusion is obvious that the (+) strain of dioecious mucors is female and the (−) strain male.—J. M. C.

Development of Pyronema.—BROWN[8] has investigated a form of *Pyronema confluens*, which he calls var. *inigneum*, in which the trichogyne does not fuse with the antheridium. He finds also that the cultural conditions for the growth of the variety appear to differ from those for the normal form in that sterilization of the substratum is unnecessary. No fusion of nuclei was observed in the ascogonium or ascogenous hyphae, the only one occurring in the asci. The fact that a species and its variety differ from one another in the distinct occurrence of the sex act and its entire absence is a striking illustration of the relation between the dependent habit and the sexual apparatus.—J. M. C.

Myxomycetes of Wisconsin.—DEAN[9] has published a descriptive list of the Myxomycetes of Wisconsin, 74 species having been identified, representing 28 genera. The relatively large genera are *Physarum* (10), *Diderma* (6), *Comatricha* (5), *Arcyria* (5), and *Trichia* (5). The descriptions are full and accompanied by critical notes.—J. M. C.

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[7] BLAKESLEE, A. F., Sexual reactions between hermaphroditic and dioecious mucors. Biol. Bull. **29**:87–89. *pls. 3*. 1915.

[8] BROWN, WILLIAM H., The development of *Pyronema confluens* var. *inigneum*. Amer. Jour. Bot. **2**:289–297. 1915.

[9] DEAN, ALLETTA F., The Myxomycetes of Wisconsin. Trans. Wisc. Acad. Sci. **17**:1221–1299. 1914.